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CHAPTER V. *The Evolution of Millstones*

FROM MORTAR TO SADDLESTONE

Early man learned that grain could be ground by pounding it with a stone pestle in a mortar. From this he advanced to rubbing the grain on a saddlestone with another stone called a metate which gave better results.

PUSH MILLS DEVELOP

Apparently from these saddlestones the push mill was developed. The surfaces of this type of mill were grooved and it was found that the shearing action was helped if the grooves were not parallel but crossed each other at acute angles. This discovery has been carried over to our day; the rollers of modern mills are so arranged for efficiency.

With better implements grinding speed increased. Then it was found that a steady supply of grain fed to the mill resulted in greater production. So the upper stone was hollowed and slotted to allow continuous feed.

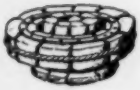
LEVER MILLS ENTER

A logical development of the push mill was the lever mill which allowed its operator to exert the force of his entire body. It increased yields so greatly that it replaced saddlestones and push mills. In fact, the lever mill was an industrial machine and caused far-reaching changes to occur in the milling of grains.

HOURGLASS MILLS & QUERNS

Next came Delian and hourglass mills. Probably these developed because the action of the lever mill in an arc suggested the greater efficiency of using a circular motion. The hourglass mill was more productive because the upper stone was suspended over the lower with both grinding surfaces running parallel.

Another development which used rotary motion was the quern which probably was developed for home grinding. In this device, too, the upper stone was suspended over the lower. As its design was refined, it gained an adjustment which allowed the grind to be changed.



FROM QUERN TO MILLSTONE

The step from quern to millstones as we know them followed logically because it was discovered that the freely-turning upper stone could be greatly increased in size and rotated on its axle by sources of much more power than could be exerted by men or animals.

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The next chapter titled: "Waterwheels and Millstones" will be published soon.

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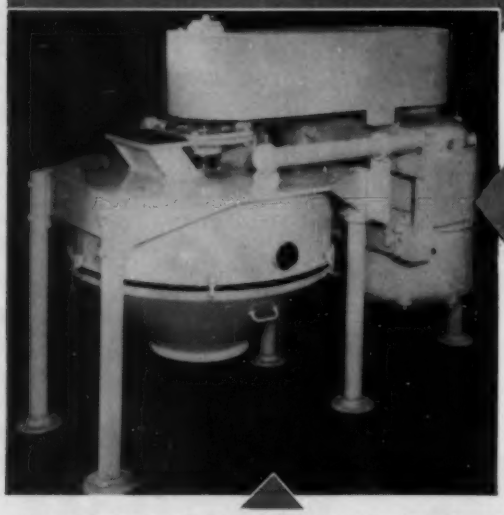

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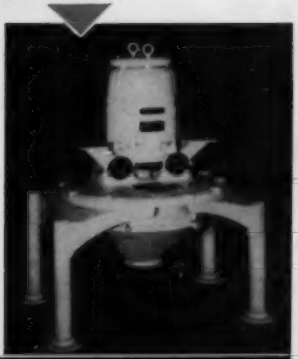

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CEREAL CHEMISTRY

VOL. 32 MAY, 1955 NO. 3

PERIPHERAL CELLS OF THE ENDOSPERMS OF GRAIN SORGHUM AND CORN AND THEIR INFLUENCE ON STARCH PURIFICATION[1]

S. A. WATSON, E. H. SANDERS, R. D. WAKELY,[2] AND C. B. WILLIAMS

ABSTRACT

Starch recovery and purification from grain sorghum are more difficult than from corn because of the occurrence in the grain sorghum kernel of a large proportion of horny endosperm, and a layer of dense cells rich in protein at the periphery of the endosperm just inside the aleurone layer. Dent corn kernels usually contain a homologous but less extensive layer than grain sorghum. The formation and abundance of such a layer is believed to be a result of protein accumulation in the endosperm of both cereals as affected by cultural and heritable influences.

Apparent resistance of the contents of the peripheral cells to fragmentation, along with the rupture of endosperm cell walls during milling, results in release of intact contents of individual cells into the starch process streams. Some of the procedures used in corn starch manufacture had to be modified for manufacture of grain sorghum starch in order to maintain quality standards.

Laboratory wet milling of different samples and varieties of grain sorghum and corn has shown that the amount of endosperm material caught on the finest bolting cloth ($44 \times 57\mu$ openings) after having passed through a coarser cloth ($60 \times 70\mu$ openings) was related to the proportion of horny endosperm in the kernels, their average protein content, and the length of the steeping period. The fine endosperm fraction from wet-milled corn was composed largely of a starchy type of cell, whereas the comparable fraction from grain sorghum was chiefly a proteinaceous type.

The endosperms of corn and of grain sorghum are structurally similar and are composed chiefly of storage parenchyma cells filled with starch granules embedded in a continuous matrix of protein. Both cereals contain regions of floury and horny endosperm. Wolf, Buzan, MacMasters, and Rist (10) reported that average cell sizes in dent corn endosperm (above scutellum) were: floury endosperm $183 \times 66\mu$; horny endosperm, $102 \times 46\mu$; sub-aleurone region, $31 \times 19\mu$. As the cells decreased in size, the starch granules became smaller and the protein matrix thicker. Hinton (2) has shown that a

[1] Manuscript received December 10, 1954. Presented at the Annual Meeting, May, 1954. This paper is a contribution from the George M. Moffett Research Laboratories, Corn Products Refining Company, Argo, Illinois.

[2] Present address: Peter Hand Foundation, Chicago, Illinois.

sub-aleurone layer in corn endosperm (flint variety) made up 3.9% of the kernel and contained 27.7% protein. Hopkins *et al.* (3) found in Burr white dent corn a so-called horny gluten layer which comprised 5.1% of the kernel and contained 22.5% protein and 7.0% oil.

Studies on the importance of these outer endosperm layers either in corn or in grain sorghum in respect to wet milling have not been reported previously. Microscopic studies of the characteristics of endosperm cells located at the periphery of endosperms in several varieties of grain sorghum and corn are described in this paper. The relationship of observed differences in peripheral endosperm properties with wet millability of grain samples is demonstrated by means of laboratory wet milling procedures.

Materials and Methods

Grains. Sources of most grain samples are acknowledged in the footnotes to the tables. Samples of Westland, Midland, Bonita, Cody × Wonder Club, and Durra grain sorghum varieties, which were examined microscopically, were obtained from the Fort Hays Branch of the Kansas Agricultural Experiment Station, Hays, Kansas. All other grain sorghum samples not otherwise acknowledged were obtained from the Texas Branch Agricultural Experiment Station No. 8, Lubbock, Texas.

Corn samples collected from pollination to maturity were obtained from a commercial field near Argo, Illinois, during the 1953 season. Representative ears were bagged and hand pollinated. Thereafter, ears were collected at 3-day intervals from pollination to 72 days after pollination. Representative kernels were cut from the ears, and fixed in mixtures of formaldehyde, acetic acid, and chromic acid known as Craf solutions.

The dry, mature samples of corn examined were obtained from sources noted in the footnotes to the tables. A sample of Argentine flint corn was obtained through commercial channels at Baradero, Argentina.

Sections. Some kernels from each sample of the corn maturation series were embedded in paraffin according to the schedule described by Sass (6), sectioned to 10μ thickness and stained with safranin and analine blue. Other kernels from this series were sectioned on the freezing microtome at 20 to 40μ thickness, stained with Sudan IV or with dilute iodine-potassium iodide solution, and mounted in glycerin.

Permanent, 10μ sections of dry, mature kernels of corn and grain sorghum were prepared for sectioning by first soaking in 5% hydrofluoric acid solution for 3 days followed by 24 hours' washing in run-

ning water. The kernels then were embedded in paraffin (6) and sectioned on the rotary microtome to 10μ thickness. Although sections were stained in some cases with safranin, analine blue, and Bismarck brown, peripheral endosperm cells were best observed when stained with dilute iodine-potassium iodide solution and mounted in glycerin. The blue color imparted to starch and the yellow color imparted to protein by iodine could be preserved for several months by storing sections in a refrigerator.

Although the 10μ sections prepared as described above were preferred for microscopic examination of peripheral endosperm, the method is relatively slow and was not suitable for surveying large numbers of kernels. For this purpose kernels were steeped and then sectioned on the freezing microtome as described previously (7). Sections usually were 40μ in thickness, although with careful handling sections of 20μ thickness occasionally could be obtained. Sections were stained with the iodine-potassium iodide solution and mounted in glycerin.

Steeping. The commercial steeping procedure has been described by Kerr (4). In steeping small lots of grain in the laboratory it is desirable to approach conditions of the commercial procedure as closely as possible, but the method must be reproducible and easily controlled. Solutions of lactic acid and sulfur dioxide in distilled water will, under certain conditions, give wet milling results equivalent to those obtained in commercial steeping practice. A two-phase system was developed which gave good results:

First Phase. The steeping medium for the first phase was composed of 1.5% (by weight) lactic acid and sufficient potassium metabisulfite to give a sulfur dioxide concentration of 0.05% (by weight) in distilled water; the mixture was adjusted to pH 3.7 with potassium hydroxide. Sodium sulfite or metabisulfite and sodium hydroxide may be used if desired. Lactic acid polymers present in the concentrated reagent should be eliminated by diluting to 20% with water and heating 12–16 hours at 90°–100°C. prior to use in the steeping medium. Fifteen hundred milliliters of the steeping medium was placed on an amount of grain calculated to contain 350 g., dry basis, in a 2-quart Mason jar which was immersed in a water bath at 52°–54°C. (120°–122°F.). The mouth of the jar was closed with a No. 12 rubber stopper fitted with two glass tubes, one of which extended to the bottom of the jar and the other just through the stopper. The tubes were connected through a pump to circulate liquid through the grain about 10 minutes each hour. A Brosites Model A0 pump was used for this purpose.

Second Phase. At the end of 40 hours the initial steeping medium was discarded and replaced with one containing 0.5% lactic acid and 0.10% sulfur dioxide and adjusted to pH 4.0. The grain was steeped in this second medium at 52°–54°C. (120°–122°F.) for 8 hours with continuous circluation. The solution then was discarded, the grain stored in a refrigerator overnight, warmed to about 45°–48°C. (80°–90°F.), and milled. Since refrigeration seemed to have a slight effect on milling results, the steeped grain was always treated in a standardized way; in the present work the grain was stored for 16–24 hours at 5°–10°C.

Milling. For complete starch isolation in the laboratory the conventional milling procedure described previously (9) generally is used. However, in the present work a shorter, more precise milling procedure was devised which provides several indices of relative millability. This method was named the "prime-starch milling procedure": Two hundred grams of the drained, steeped grain were weighed to the nearest kernel into a 250-ml. beaker. A 50- to 100-g. sample was retained for moisture determination. The weighed grain sample and 250 ml. of water were placed in a Waring Blendor which had the cutting edges of the agitator blades ground to give a blunt edge for striking the grain. The blendor motor was operated through a variable transformer set at 85 volts. Grinding was continued 1 minute for corn and 1.5 minutes for grain sorghum. The ground mass and washings from the bowl were poured onto No. 5028 nylon bolting cloth fixed to a Kahn or other reciprocal type of shaker. This bolting cloth has openings averaging $60 \times 70\mu$, which is approximately equivalent to U.S. Standard sieve No. 230. The residue was washed on the shaker with about 500 ml. of water from a wash bottle in order to remove all freed starch. A thorough and uniform washing of the residue on the shaker had to be accomplished without the use of unduly large quantities of water. The residue on the nylon screen was discarded. The filtrate was collected and quantitatively passed over a No. 25 silk bolting cloth stretched on a shaker frame. The residue was thoroughly washed free of loose starch in a standardized manner with about 100 ml. water from a wash bottle. The openings of the No. 25 silk average $44 \times 57\mu$ although cloth from different manufacturers may differ somewhat as to dimension of the openings. A 325-mesh U.S. Standard brass sieve (openings average $44 \times 44\mu$) can be used in place of the No. 25 silk with approximately the same results. The severity of washing the residue on the screen will influence results obtained. The washed residue retained on the No. 25 silk was quantitatively washed from the silk into a beaker and made up to 100 ml. This fraction was

composed chiefly of peripheral endosperm cells. The particles were kept suspended in the water while a 50-ml. sample was removed with a pipet for determination of insoluble dry substance, and a 25-ml. sample transferred to a Kjeldahl flask for determination of nitrogen. The volume of the slurry obtained by filtration through the No. 25 silk (prime-starch fraction) was measured (about 1000 ml.). The prime-starch fraction was thoroughly suspended and 25-ml. aliquots were removed for determinations of insoluble dry substance and protein. The insoluble dry substance was most conveniently determined with a weighed porous-bottomed filtering crucible of glass or porcelain. The crucible with its filter cake was dried overnight at 50°–60°C. followed by 4 hours in a vacuum oven at 115°C. Yields of the prime-starch fraction and the residue on the No. 25 silk were calculated as percentage of the steeped grain dry substance.

Analyses. Moisture in steeped grain was determined on a whole-kernel sample. About 25–30 g. of wet grain were weighed into an aluminum moisture dish (77 mm. high $\times$ 25 mm. diameter) and dried 8 hours in an air oven at 60°–100°C. and then in a vacuum oven 24 hours at 115°C. Nitrogen was determined by the A.O.A.C. official Gunning procedure (1) and protein calculated as nitrogen $\times$ 6.25. Alcohol-soluble protein was determined by adding 20 ml. of 50% isopropyl alcohol to 1.0 g. of the sample contained in a test tube and the mixture agitated for 15 minutes at 50°C., centrifuged, and the supernatants decanted. Each sample was extracted three times, and the supernatants were combined and analyzed for nitrogen.

Results and Discussion

Dense Peripheral Endosperm Cells in Grain Sorghum. The function of steeping is to soften the protein matrix and endosperm cell walls for easy rupture to release starch during milling. If the protein matrix is not thoroughly softened, starch granules may not be released and milling may result in the formation of pieces of endosperm like the S (starchy type) particles in Fig. 1C and 1D. These particles were observed in off-grade grain sorghum starch. They are composed of starch granules bound together by intact protein matrix. The smooth edges of some of the particles indicated that they might be intact contents of individual endosperm cells, although some particles had one irregular edge. Average size of these particles was $50 \times 62\mu$ with a range of $36 \times 45\mu$ to $75 \times 120\mu$. Particles of the other type observed (P, Fig. 1) appeared to be composed almost entirely of protein, although a few small starch granules showed up as blue spots in a yellow mass when the sample was stained with iodine-potassium

iodide solution. The outline of these particles was smooth, suggesting that they were intact cells. Judging from their appearance under high magnification they were of endosperm origin. No similar type of particle had been noticed previously in fractions obtained by the wet milling process. Average size of these particles was $56 \times 81\mu$ with a range of $48 \times 54\mu$ to $60 \times 111\mu$. Many of the particles were more or less flattened as shown in Fig. 1A and 1B. When off-grade grain sorghum starch was passed over a 325-mesh screen, 0.2 to 0.4% was re-

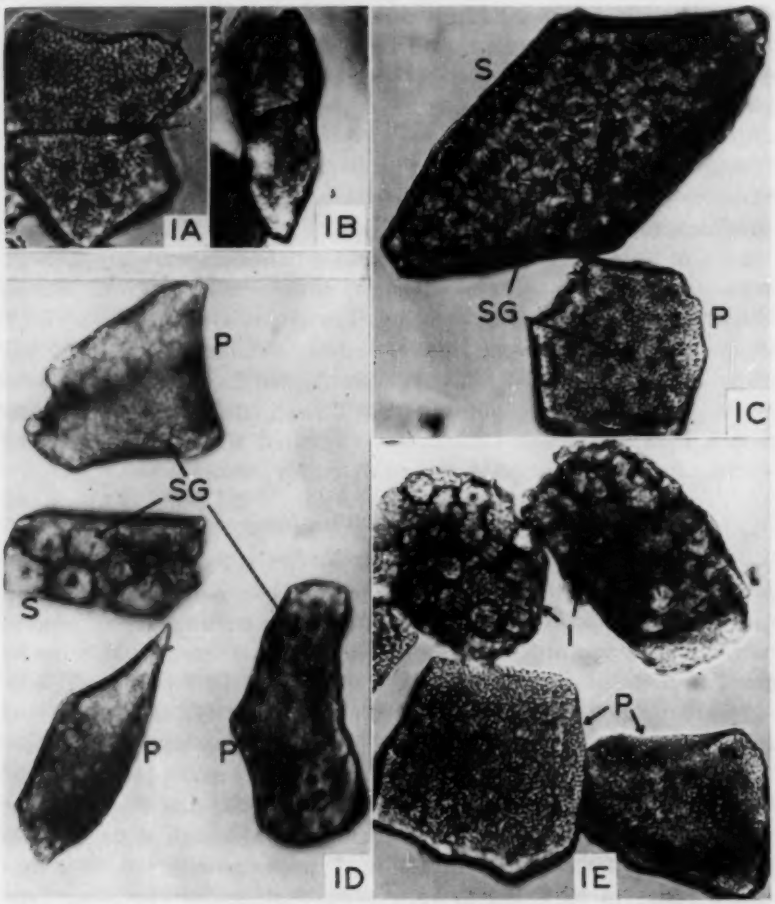

Fig. 1. Contents of endosperm cells recovered from off-grade Martin grain sorghum starch on 325-mesh screen: P, dense peripheral endosperm cell contents (proteinaceous type); S, starchy type from horny endosperm; I, particles with some characteristics of both starchy and peripheral types; SG, starch granules. 1A and 1B, broadside and edge views, respectively, of the same particle apparently composed of two adjacent peripheral cells; 1C, 1D, and 1E, representative endosperm cell contents. 360 ×.

tained on the screen with protein contents ranging from 25 to 45%. Numerically, the proteinaceous type of particle constituted one-half to three-fourths of the particles in the sample.

In order to verify conclusions from microscopic observations of the proteinaceous particles, several other lines of evidence were pursued.

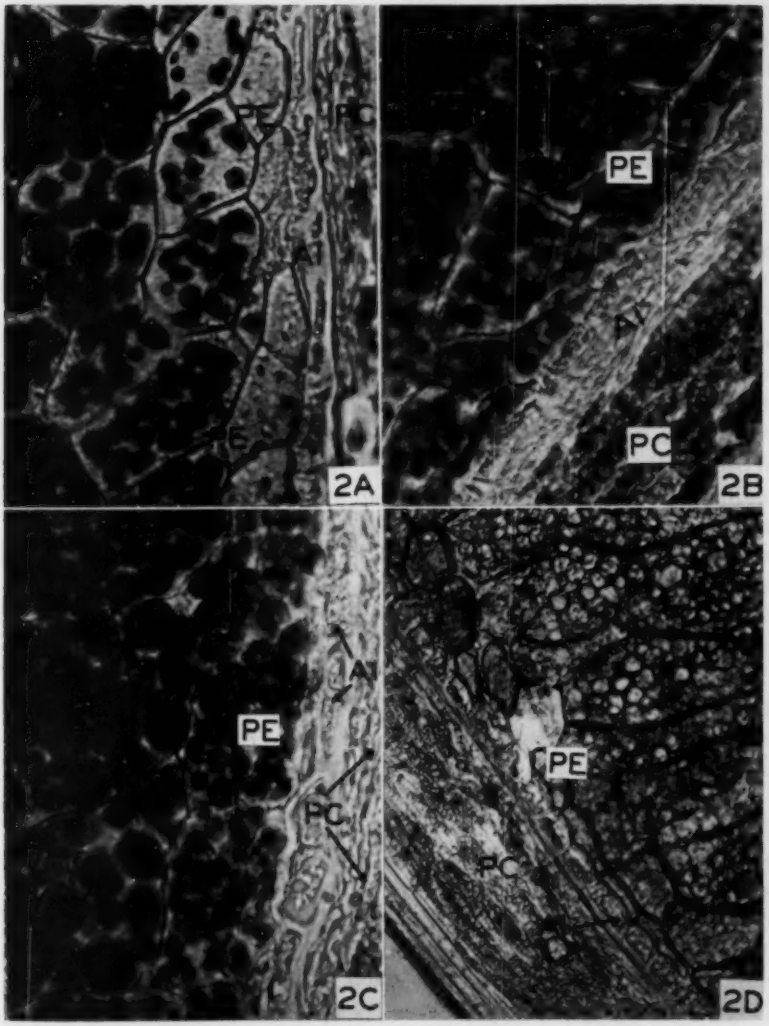

Fig. 2. Longitudinal sections of grain sorghum endosperms: 2A, var. Combine Kafir 54T, 10μ, 560 ×; 2B, var. Martin (Lubbock, Texas), 10μ, 560 ×; 2C, var. Martin (Arizona), 10μ, 570 ×; 2D, var. Martin (Corpus Christi, Texas), 20μ; 195 ×. Al, aleurone layer; PC, pericarp; PE, peripheral endosperm.

Since the alcohol-soluble protein, kafirin, is characteristic of grain sorghum endosperm, as zein is characteristic of corn endosperm, analysis of the particles for their kafirin content helped to identify them with endosperm. The protein in the wet milling fraction of grain sorghum known as gluten was found to be 83% soluble in 50% isopropyl alcohol, while the protein in particles collected on a 325-mesh screen from off-grade grain sorghum starch was 92% soluble. Since gluten is more or less purified endosperm protein, the similar kafirin content of these two fractions indicated they were from the same region of the kernel.

Numerous steeped sorghum kernels were sectioned on the freezing microtome and raw, whole kernels sectioned on the rotary microtome. Almost every kernel examined had a layer of cells in the endosperm just inside the aleurone (Fig. 2) which appeared to be identical with the proteinaceous type of particles recovered from starch. This layer, which was designated the dense peripheral endosperm layer, was about 1 cell in thickness just above the germ and on the side of the kernel at the level of the mesocotyl. At the crown and at the base of the kernel the layer became about 2 to 3 cells in thickness. The layer of cells closest to the aleurone appeared to be flattened tangentially, while those in the second or third row were often more or less isodiametric. Cell contents were composed of granular protein in which small starch granules were embedded. Different samples of the same variety of grain sorghum showed considerable variation in both thickness of the peripheral layer and density of protein in the individual cells. Sections of three samples of Martin variety are shown in Fig. 2B, 2C, and 2D, listed in the order of increasing thickness and density of the peripheral endosperm. Figure 2A is a 10μ section of Combine Kafir 54 in which the thickness of the peripheral layer is about the same as that of the Martin sample in Fig. 2D. Many varieties of grain sorghum were examined and all contained the dense peripheral endosperm layer. Despite great variations among samples of any one variety, there appeared to be some real varietal differences. The varieties Shallu, Combine Kafir 54T, Combine Kafir 60, Martin, Westland, Midland, Redbine 66, and Cody $\times$ Wonder Club had relatively thick, dense peripheral layers, while the varieties Plainsman, Caprock, Redbine 60, Texioca 54 (waxy), Double Dwarf White Sooner (waxy), Double Dwarf Yellow Sooner, Durra, Hegari, Bonita, and Feterita had relatively less pronounced peripheral endosperm layers. A detailed study of the developmental morphology of dense, peripheral endosperm in several varieties of grain sorghum was reported by Sanders (5).

Figure 2D illustrates what is thought to happen to the peripheral

endosperm during the milling of steeped sorghum kernels. The kernel from which the section was obtained was well steeped, as indicated by loss of most of the starch from the floury endosperm (not shown). Gentle manipulation of the cover slip resulted in dislodgement of the dense peripheral cells. The cell walls were apparently so softened during steeping that mechanical manipulation loosened them from the more rigid cell contents. The same action probably occurs in the wet milling of steeped grain. The crushing action of the mill liberates starch from most endosperm cells, but the small, dense peripheral cells apparently resist fragmentation owing to their high protein and low starch content. A study of a commercial grain sorghum wet milling system indicated that peripheral cells were liberated in the coarse degermination step as well as in the fine grinding step.

Wet Milling Evaluation of Dense Peripheral Endosperm in Grain Sorghum. In order to demonstrate the effect of differences in thickness of dense peripheral endosperm in grain sorghum, six samples of grain known to differ in this respect were steeped 48 hours by the two-phase laboratory procedure. Duplicate samples from each steep were milled by the prime-starch milling procedure. Results given in Table I show the wide range in milling properties that may exist among grain samples of different varieties as well as among different samples of the same variety. The residue on No. 25 silk, composed largely of

TABLE I

COMPARATIVE MILLING OF DIFFERENT VARIETIES OF STEEPED GRAIN SORGHUM
(Averages of duplicate millings)

Variety and Description	Total Protein	Steeped Moisture	Prime-Starch Fraction		Residue on No. 25 Silk	
			Yield	Protein	Yield	Protein
	%d.b.	%	%d.b.	%d.b.	%d.b.	%d.b.
Martin (Arizona)[a]	11.3	47.9	64.3	8.3	0.14	34.9
Martin (Lubbock)	11.0	45.0	61.4	5.3	0.33	36.3
Martin (Corpus Christi)[b]	13.7	41.0	58.1	8.9	0.55	37.5
Texioca 54 (waxy)	11.3	47.4	61.8	6.6	0.40	28.7
Feterita, Double Dwarf White	13.8	44.5	60.0	8.1	0.44	27.8
Shallu, Double Dwarf Early	14.4	39.4	49.3	8.8	0.49	38.5
Combine Kafir 54T	13.4	41.2	43.4	7.6	0.86	36.4

[a] Purchased from Advance Seed Co., Phoenix, Arizona.
[b] Commercial grain. All other samples were obtained from the Texas Branch Agricultural Experiment Station No. 8, Lubbock, Texas.

peripheral endosperm cells, gave values ranging from 0.14 to 0.86% of the grain dry substance, indicating wide differences in thickness of the dense peripheral layer among the seven samples. The yield of prime starch, total kernel protein content, and steeped grain moisture are indicative of the amount and hardness of the horny endosperm. On all counts the Combine Kafir and Shallu samples were the hardest and had the thickest layers of dense peripheral endosperm. Figure 2A shows the peripheral endosperm layer in Combine Kafir 54T. Shallu was very similar. The Martin sample from Corpus Christi was also quite hard and had a relatively thick peripheral endosperm layer (see Fig. 2D). Both laboratory and manufacturing experience indicated that samples of Martin variety grown in the Coastal Bend area were generally harder and higher in protein content than samples of this variety from other parts of Texas. The sample from Lubbock (see Fig. 2B) was intermediate in milling properties, whereas that from Arizona was unusually soft for the Martin variety. The latter sample gave an unusually low yield of residue on No. 25 silk and upon microscopic examinations of thin kernel sections it was found to be almost completely devoid of a dense peripheral endosperm layer (see Fig. 2C). Feterita, which is related to Hegari variety, is usually considered as having soft kernel properties. The sample examined here was intermediate. However, the lower protein content of the residues on No. 25 silk for both the Feterita and Texioca samples indicates that the peripheral endosperm may not have been as extensive as in the other varieties. The high moisture content and high prime-starch yield of the Texioca and Arizona Martin samples indicated unusually soft kernel characterstics. Waxy varieties of grain sorghum, represented herein by Texioca, previously have been shown to yield more starch having a higher purity than was the case with regular sorghum varieties (8).

The amount of the residue on No. 25 silk decreased with increased length of time that the grain sorghum was steeped. This effect is shown for two samples of Martin grain sorghum in Table II. Sample No. 2B had the thinnest layer of dense peripheral endosperm of the two and showed almost a minimum amount of residue on No. 25 silk by the end of 16 hours' steeping. Sample No. 2D from Corpus Christi, the same sample as given in Table I and shown in Fig. 2D, gave a high yield of residue on No. 25 silk and a long steeping time was required. The increasing protein content that accompanied decreasing yield of the residue on No. 25 silk was believed to be the result of a concentration of dense peripheral cells in the fraction. Longer steeping time probably resulted in more complete fragmentation during the milling

TABLE II

EFFECT OF LENGTH OF STEEPING PERIOD ON NO. 25 SILK RESIDUE

Variety and Where Obtained	Total Kernel Protein	Residue on No. 25 Silk							
		Yield				Protein			
		Hours Steeped:				Hours Steeped:			
		4	8	16	54	4	8	16	54
		%d.b.	%d.b.	%d.b.	%d.b.	%d.b.	%d.b.	%d.b.	%d.b.
Martin No. 2B (Lubbock)	10.8	0.51	0.37	0.27	0.25	28.2	34.9	40.3	41.7
Martin No. 2D (Corpus Christi)	13.7	1.26	1.01	0.86	0.60	25.3	29.6	33.2	38.2

of regular endosperm cells but had no effect on the dense peripheral cells. Other steeping experiments have shown that any unfavorable condition which inhibits or retards the steeping of the kernel results, on milling, in a higher yield and lower protein (higher starch) content of the residue on No. 25 silk.

Dense Peripheral Cells in Corn Endosperm. The results obtained with grain sorghum suggested a reexamination of the corn kernel and corn wet milling fractions for similar types of cells. Endosperm particles in corn starch have not been recognized as a problem. However, when corn wet milling fractions were examined after passing successively over No. 5028 nylon screen (or 230-mesh wire screen) and then over a 325-mesh wire screen (or No. 25 silk), the particles shown in Fig. 3 were obtained. Ninety per cent of these particles were similar to the starchy type (S) from grain sorghum but the remainder were the proteinaceous type (P). The total amount of such particles in corn fractions was much smaller than in grain sorghum. They were composed of starch granules held together by a protein matrix. The smooth sides indicated that they were parts of intact endosperm cell contents which had apparently broken away from cell walls in the same manner as has been described for grain sorghum. The average size of all particles was $50 \times 87\mu$ in a range of $36 \times 66\mu$ to $60 \times 120\mu$. From a comparison with measurements made by Wolf *et al.* (10) this size range indicates that the particles originated in the outer horny endosperm as well as in the region designated as sub-aleurone. A few small, dense peripheral cells were found in the fraction that passed through the 325-mesh screen.

Samples of mature dent corn were sectioned by the paraffin embedding method and examined microscopically. Most of the peripheral endosperm region of an average sample of commercial corn looked like the section of yellow dent shown in Fig. 4A (grown near Argo, Illinois). However, in most kernels a small region could be located

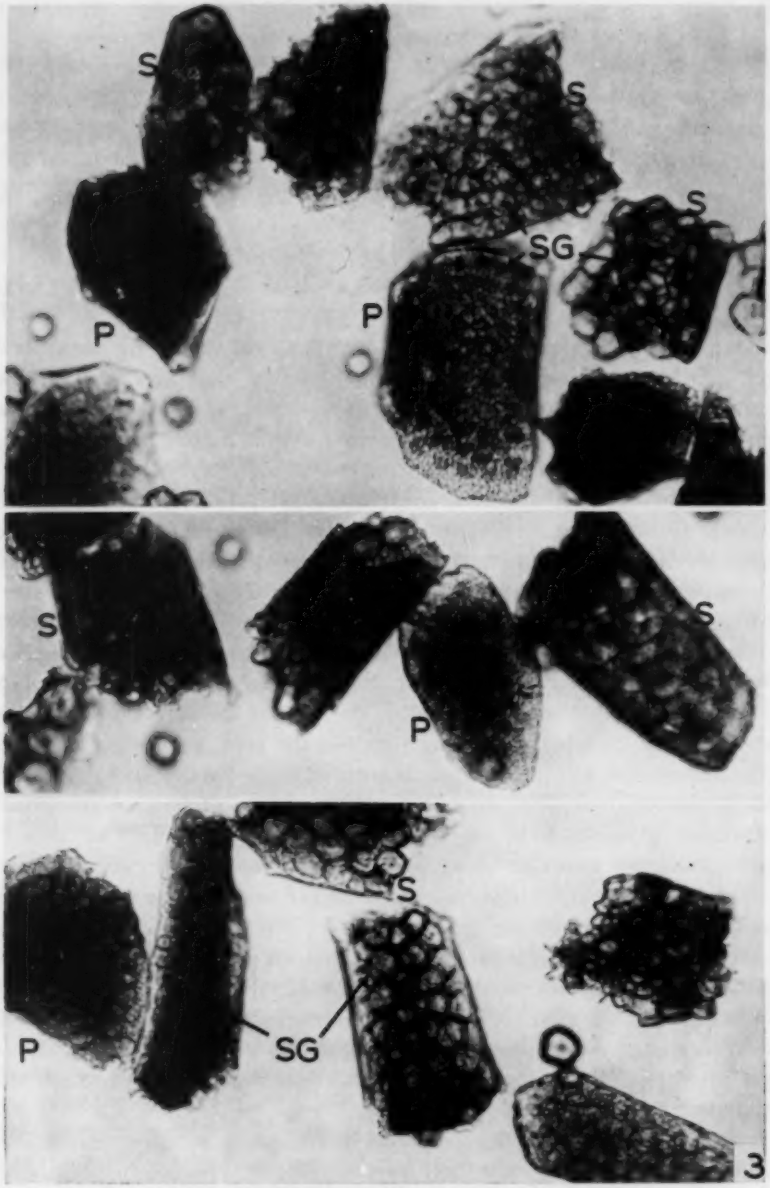

Fig. 3. Contents of endosperm cells recovered from corn starch process stream on 325-mesh screen: P, dense peripheral cell contents (proteinaceous type); S, starchy type from horny endosperm; SG, starch granules. 360 ×.

similar to that shown in Fig. 4B and a smaller, denser region in the crown or its margin (Fig. 4C). The latter two photomicrographs were taken from a 10μ section of a sample of dent corn of medium high protein content (10.5% protein, grown at Clinton, Illinois). The cells shown in Fig. 4B were located on the side of the kernel slightly

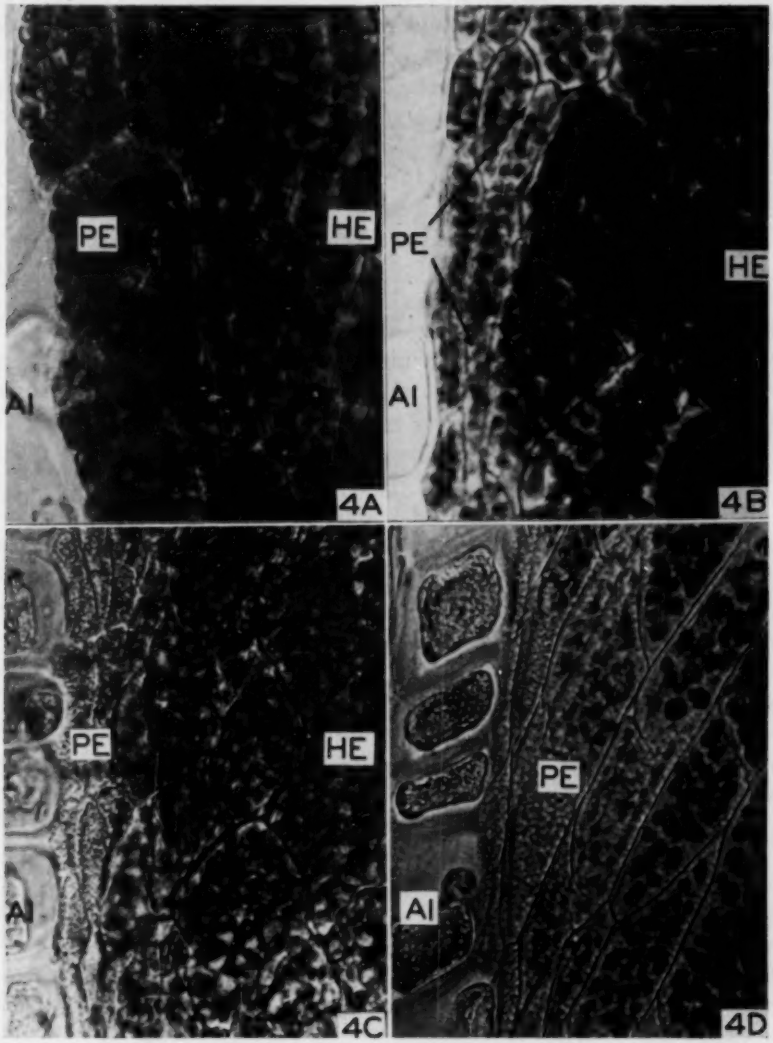

Fig. 4. Longitudinal 10μ sections of corn endosperms: 4A, var. yellow dent (Argo, Illinois), side of kernel; 4B, var. yellow dent (Clinton, Illinois), side of kernel just below crown; 4C, same kernel, crown; 4D, Illinois High Protein strain, side of kernel just below crown. Al, aleurone layer; PE, peripheral endosperm; HE, horny endosperm. 535 ×.

below the crown. These cells were similar to the peripheral endosperm cells in grain sorghum but were often more flattened. These observations indicated that the occurrence of dense peripheral endosperm was related to the protein content of the endosperm in corn as was the case with grain sorghum. This was verified upon examination of 10μ kernel sections of the Illinois High Protein strain (19.3% total protein). Figure 4D shows the very dense peripheral cells found in this corn

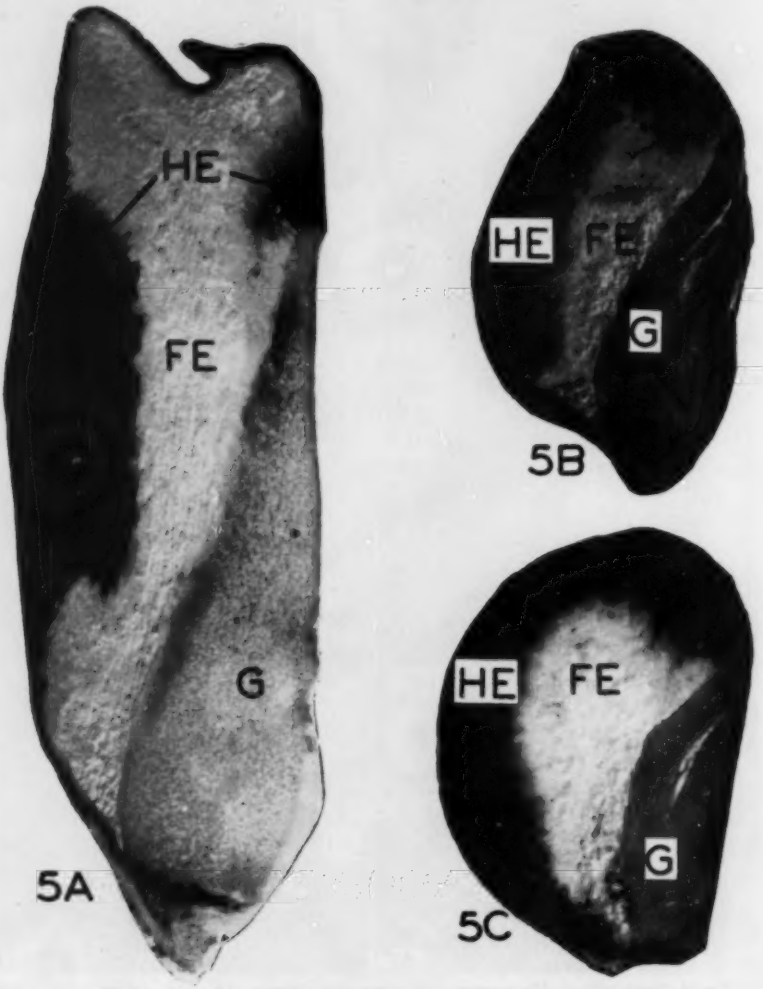

Fig. 5. Distribution of horny and floury endosperm regions in corn and grain sorghums: 5A, dent corn, 9 ×; 5B, grain sorghum, var. Shallu, 15 ×; 5C, grain sorghum, var. Martin, 15× . FE, floury endosperm; HE, horny endosperm; G, germ.

near the edge of the crown. On the whole they are even more elongated than in grain sorghum. The dense peripheral endosperm layers of a high protein dent corn hybrid (12.6% protein) and Argentine flint (11.2% protein) were intermediate in thickness.

Although the presence of dense peripheral endosperm appears to be the result of greater nitrogen accumulation in the endosperm, the grain sorghum kernel seems on the whole to have a greater tendency to produce this type of cell structure. Because the problem of starch purification in corn wet milling has never been as acute as it has been with grain sorghum, it was of interest to investigate the cause of the difference between these two cereals. Some of the differences in properties between corn and grain sorghum have been discussed elsewhere (8). Comparison of the gross structural features in Fig. 5 reveals some obvious differences that would affect this problem. The grain sorghum kernel is about one-tenth the size of the corn kernel and hence the ratio of endosperm surface area to endosperm weight is much greater in grain sorghum than in corn; therefore, the effects of peripheral endosperm on milling results would be proportionally greater. Furthermore, the grain sorghum kernel contains a much greater proportion of horny endosperm than does dent corn. Horny endosperm is the source of the starchy type of endosperm particle "S" shown in Figs. 1 and 3.

The greater tendency of grain sorghum than corn to form the dense peripheral endosperm cells has not been explained. To this end corn kernels were collected at 3-day intervals from pollination to 72 days after pollination and sectioned, stained, and examined in comparison with the developmental studies on grain sorghum (5). Development was parallel in both cereals up to the time cell divisions ceased. Cell divisions ceased first at about 3 weeks after pollination at the crown region above the germ; cessation of meristematic activity travelled as a wave over the crown and down the sides of the kernel. By the fifth week all cell division had stopped and the last cells formed at the base had begun to mature. Maturation, accompanied by starch deposition, followed cessation of cell division in the same wavelike pattern. The cytoplasm of cells in the sub-aleurone region was more dense at first than the cytoplasm in the aleurone. However, as cells matured and starch was deposited in them, the sub-aleurone ceased to be a distinct layer. This maturation process continued until about the tenth week. By this time peripheral cells were not different from other endosperm cells except in size and in the size of their starch granules. In grain sorghum the maturation of peripheral endosperm cells was completed soon after the cell divisions ceased and did not include the period of

starch deposition observed in corn. The physiological reasons for this difference in behavior are not known.

Wet Milling Evaluation of Differences in Peripheral Endosperm Layer in Corn Varieties. The effect of variations in endosperm properties of different samples of corn when wet milled was investigated, using the prime-starch milling procedure. Samples selected for study had a range of 5.8 to 19.3% total protein and included five dent corns and one flint corn. The horny endosperm area in longitudinal cross-section (7) varied from 7 to 27%. These samples were steeped 48 hours by the two-phase steeping method. Results are given in Table III. The yield of residue on No. 25 silk and the protein content of prime-starch fraction appeared to be correlated with total protein content of the grain and with the percentage of horny endosperm area if the Argentine flint corn and the Illinois High Protein strain were omitted. Because of a higher proportion of horny endosperm in the Argentine flint corn, it was expected that it would exhibit poorer wet milling characteristics than were actually obtained. However, its protein matrix and dense peripheral layer when observed under the microscope appeared to be only a little thicker than homologous areas in the dent corn sample from Clinton, Illinois (Fig. 4B and 4C). The Illinois High Protein strain had the poorest wet milling characteristics. This apparently was due to the presence of a thick, dense peripheral endosperm layer (see Fig. 4D) and to an especially thick protein matrix throughout both the horny and floury endosperm regions. Comparison of these

TABLE III

COMPARATIVE MILLING OF DIFFERENT VARIETIES OF CORN
(Average of duplicate millings)

Variety or Strain	Total Protein	Horny Endosperm Area	Steeped Moisture	Starch-Protein Fraction		Residue on No. 25 Silk	
				Yield	Protein	Yield	Protein
	%d.b.	%	%	%d.b.	%d.b.	%d.b.	%d.b.
Ill. Low-Protein[a]	5.8	7.0	54.5	60.3	3.8	0.04	16.2
Yellow Dent (Argo, Ill.)[b]	9.3	12.0	48.3	61.9	6.2	0.08	18.9
Yellow Dent (Clinton, Ill.)[c]	10.5	24.0	46.5	59.5	5.3	0.19	15.2
High Protein Yellow Hybrid[d]	12.6	34.0	47.5	61.0	9.7	0.29	27.8
Ill. High-Protein	19.3	34.0	47.4	46.5	19.6	1.27	26.4
Yellow Flint, Argentina	11.2	47.0	43.3	58.4	9.2	0.17	25.3

[a] Obtained from Illinois Agricultural Experiment Station, Urbana, Illinois.
[b] Pioneer Hybrid No. 349 — medium soil nitrogen.
[c] Thorpe Seed Co.; pollen parent ears from Funk G-94 hybrid; high soil nitrogen.
[d] Funk Seed Co. experimental hybrid grown at Urbana, Illinois.

same corn varieties by the section-scoring method, reported previously (7), showed about the same order of millability as was obtained by the prime-starch procedure.

The effect of length of the steeping period on millability of corn determined by the prime-starch milling procedure, as shown in Table IV, was similar to the effects observed with grain sorghum. These

TABLE IV

WET MILLING PROPERTIES OF CORN FROM SUCCESSIVE STEEPS IN A COMMERCIAL COUNTERCURRENT BATTERY

Steep No.	Hours Steeped	Moisture	Prime-Starch Fraction[a]		No. 25 Silk Residue[a]	
			Yield	Standard Deviation	Yield	Protein
		%	%d.b.		%d.b.	%d.b.
11	41.0	44.4	64.0	±0.90	0.05	28.3
10	37.5	45.6	63.7[b]	0.70	0.04[b]	34.3
9	34.0	45.6	63.0	0.32	0.05	28.9
8	30.0	45.1	59.7	0.69	...	...
7	24.0	43.5	61.0[c]	...	0.07[c]	29.0
6	20.0	44.9	56.2	0.12	0.22	29.6
5	17.0	44.0	52.5	0.60	0.58	35.7
4	13.5	35.4[c]	40.9[c]	...	0.39[c]	39.8
3	10.0	41.5	36.6	0.57	0.38	36.5
2	6.5	35.5	31.3[c]	...	0.17[c]	39.8
1	3.0	32.8	19.7[b]	0.50	0.12[b]	38.5

[a] Duplicate determinations by each of three operators except where noted.
[b] Duplicate determinations of one operator.
[c] Single determination.

samples were removed within a few minutes of each other from successive steeps of a commercial countercurrent steeping battery. The samples in the different steeps were not necessarily from the same source. The yield of residue on No. 25 silk was low from the fully steeped corn, but as the corn was more incompletely steeped the yield of this fraction increased. However, in steeps ranging from 17 hours down to 3 hours the corn was apparently so hard that the amount of small fragments made in the milling declined. The protein content of the residue on No. 25 silk did not change significantly, indicating that particles were all of one type. The yield of the prime-starch fraction was high for the corn steeped 41 to 34 hours, showed a small drop for the 24- and 20-hour steeped corn, but then decreased fairly steadily as the steeping time decreased. As was found with grain sorghum, the yield of the residue on No. 25 silk is increased by unfavorable steeping conditions.

Quality requirements for corn starch allow a maximum content of about 0.35% protein, but in the manufacture of grain sorghum starch

the characteristics of the sorghum endosperm are such that this value has been difficult to attain. However, information from the experiments reported herein has made possible manufacturing modifications which have yielded grain sorghum starch of desired quality.

Endosperm particles in finished corn starch have not been an important factor in starch quality because, as shown above, corn has a relatively small proportion of dense peripheral cells in the endosperm. However, emphasis on higher protein values in corn as a feedstuff through the use of improved hybrids and an increase in the use of nitrogenous fertilizers may be altering corn in the direction of a higher proportion of horny endosperm and dense peripheral endosperm layers. Furthermore, the changes in commercial methods of milling and of starch-protein separation may tend to increase the problem of endosperm fragments in starch. In centrifugal or flotation methods of separation, more of the endosperm particles are likely to remain in the starch stream than when tables are used for starch-protein separation. This means that greater care may be needed in manufacturing operations to remove endosperm fragments, for example, by more efficient screening.

Acknowledgments

Work on the two-phase steeping procedure was carried out by Mr. Yoshiro Hirata.

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COMPARISONS BETWEEN MALT AND BARLEY GUMS ISOLATED BY VARIOUS PROCEDURES[1]

W. O. S. Meredith and J. A. Anderson

ABSTRACT

Gums were prepared from raw and enzyme-inactivated barley and kilned malt. Enzyme inactivation, by boiling alcohol or papain extraction, had practically no influence on hexose-pentose ratios of barley or malt gums, but decreased yield and increased solution viscosity of gum. The malt gum-degrading enzyme systems were more active than those in barley, but responded in the same way as the barley enzymes to boiling alcohol and papain, and at least two enzyme systems are indicated.

Both barley and malt contain insoluble material of the same composition as the respective soluble gums; this insoluble material is attacked by one or more enzymes that render it soluble during aqueous extraction of barley or malt.

Malt gum is much higher than barley gum in absolute and percentage amount of pentose and lower in glucose. The study provides information on enzymes active during malting that require further investigation.

The change from barley to malt by germination is accompanied by many alterations in physical structure and biochemical properties of various components of the kernel. The extent of many of these has been determined (1, 5), and the total effect is generally known as modification. However, some of the changes have not been adequately investigated and are ill-defined. An outstanding example is the lack of information on the course of dissolution of cell wall material during malting. This dissolution is required to expose cell contents to attack by the appropriate enzymes and is widely regarded as a chief characteristic of the malting process (15). The so-called barley gums are considered to be important components of the cell wall, and with their isolation in undegraded condition, by Preece and Mackenzie (20) and in this laboratory (13), the way has been cleared for studying malt gums and measuring the gross change from barley to malt. Eventually, it should be possible to use this information, together with related information on enzymes, to provide a more detailed picture of the mechanism of modification.

Preece and his group are also engaged in studies of barley and malt gums and the enzymes that degrade them (15–21), and there is considerable agreement between their results and ours (2, 3, 4, 12, 13), even though they studied two-rowed barleys (17–21) and we examined only samples of a six-rowed variety, Montcalm, from several crop years

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(12, 13). The agreement both within and between the results of the two groups suggests that, despite the rather limited number of samples examined (particularly in the Canadian series), hypotheses on the fundamentals of gum characteristics may be developed on reasonably secure premises.

The possible practical application of results of basic research on gums and enzymes to evaluation of malting behavior for different varieties has been investigated by Preece *et al.* (17, 18). Although their results express in quantitative forms some of the relations between barley gums and enzymes that degrade them, and some features of malting behavior, the latter was not readily predicted. This further emphasizes the need for more basic information on barley and malt gums.

The present study was based on isolation of malt gums by three procedures involving enzyme inactivation procedures previously used to obtain barley gums in undegraded conditions (13). Comparisons of properties and composition have also been made between the malt gums and comparable gums from the barley from which the malt was made. Major differences between the barley and malt gums should provide a basis for detailed studies of gum and enzyme development in malt at various stages of growth.

Materials and Methods

Gums were prepared from commercially grown Montcalm barley from the 1952 crop and from commercial malt made from the same lot of barley.

The basic methods for isolation of barley gum have already been recorded (13) and may be summarized as follows: Barley was ground in a Wiley mill and malt in a Miag (cone type) mill. Alcohol treatment consisted of boiling the grist with five parts of 85% ethanol under reflux for 30 minutes. Raw and alcohol-treated grists were extracted with water or a 0.025% solution of papain at 20°C. to provide four types of extract. The papain was removed by trichloracetic acid and gum was precipitated by pouring the liquid into three volumes of ethanol. Four gums were thus obtained from barley and four from malt. The gums were prepared in duplicate and hydrolysates and analyses were also made in duplicate.

Viscosity measurements were made in Ostwald viscosimeters using 1% aqueous solution of gum (13). Samples of the gum were hydrolyzed in sealed tubes with *N* sulfuric acid, and sugars in the hydrolysates were identified by chromatography as described previously (13). Quan-

titative measurements of the individual sugars were made by the phenol-sulfuric acid method of Dubois *et al.* (6).

Results and Discussion

Effect of Treatment on Gum Yield. Data showing the effect of treatment on gum yield are given in Table I. All treatments of the

TABLE I

YIELD OF GUM FROM GRAIN

Treatment	Yield	
	Barley	Malt
	%	%
None	2.38	2.48
Papain	1.78	0.65
Alcohol	1.00	0.31
Alcohol and papain	1.40	0.54

barley designed to inactivate enzymes caused reduction in gum yield. The alcohol treatment produced the lowest yield, and the combination of alcohol and papain resulted in a yield that was intermediate between those from the alcohol treatment and the papain treatment. These results are practically identical with those obtained previously in this laboratory (13). We consider that the combination of alcohol and papain treatment provides a product that is closely similar to the naturally occurring water-soluble gum (13).

The effects of treatment on malt are very similar to those for barley, and again the alcohol treatment produced the lowest yield. Yield of gum from untreated malt was essentially the same as that from untreated barley, but all treatments reduced malt gum yield much more than barley gum yield. Gum yields from treated malt were about one-third of those from corresponding treatments of barley.

The reductions in yield suggest that both barley and malt contain material in an insoluble form that resembles the initially soluble gum. This material is degraded during extraction by enzymes present in both barley and malt to yield a soluble product. However, as yields of gum from both barley and malt are different when either papain or boiling alcohol is used to inactivate enzymes, there is a suggestion that two enzyme systems are involved in degradation of the insoluble material. One is inactivated by boiling alcohol and one by papain.

Effect of Treatment on Viscosity of Aqueous Solution of Gum. The viscosities of 1% aqueous solution of the gum are given in Table II.

TABLE II

VISCOSITY OF AQUEOUS SOLUTIONS OF GUMS

Treatment	Viscosity, C.P.	
	Barley Gum	Malt Gum
None	1.8	1.4
Papain	2.3	3.2
Alcohol	332.0	6.4
Alcohol and papain	600.0	4.2

The highest solution viscosity for barley gum was obtained from the combined treatment with boiling alcohol and papain. The solution of this gum was stable. Alcohol alone produced gum with intermediate solution viscosity, but the solution was unstable, i.e., viscosity decreased slowly but steadily over a 24-hour period. The gum from untreated barley was lowest in solution viscosity, and that from papain-treated barley was only slightly higher in solution viscosity.

All the malt gums were relatively low in solution viscosity. The highest value was obtained from malt treated with alcohol, and all treatments produced gum of higher solution viscosity than that from the untreated malt. All the malt gums were stable in solution, but were of relatively low solution viscosity, especially when compared with the value for gum from barley treated with both boiling alcohol and papain. As solution viscosity is a measure of molecular size, all malt gums appear to be much lower in molecular weight than the undegraded barley gum.

The results for viscosity and stability of solutions of barley gums confirm and amplify the suggestion made in the previous section and in previous papers (4, 13) that endo- and exo-enzyme systems are involved in degrading insoluble material to soluble products and also in degrading the initially soluble barley gum[2]. The endo-enzyme system in barley degrades the gum by cleaving internal linkages (4) and is inactivated by boiling alcohol. The exo-enzyme system cleaves external linkages (4) and slowly reduces molecular size, thus causing the steady

[2] Since this paper was submitted, Preece, I. A., Aitken, R. A., and Dick, J. A. (J. Inst. Brewing, 60: 497-507. 1954) have expressed similar views and have adopted our terminology (4).

decrease in viscosity of solutions of gum from alcohol-treated barley. This enzyme is presumably inactivated by papain.

The data for solution viscosity of the malt gums have little to offer in support of this suggestion, as the malt gums appear to be much more disaggregated than the barley gums. However, gum from untreated malt appears to be in the lowest state of molecular complexity.

Effect of Treatment on Pentosan Content of Gum. Arabinose and xylose were determined separately in hydrolysates of each gum, and all ratios of these sugars were about 1 : 2. The values were added to provide a single figure for pentose content. Results for each gum are given in Table III.

TABLE III

PENTOSE CONTENT OF GUMS

Treatment	Pentose	
	Barley Gum	Malt Gum
	%	%
None	17.9	73.0
Papain	14.4	72.5
Alcohol	17.0	85.0
Alcohol and papain	20.5	89.0

Among the barley gums, the gum from the combination of alcohol and papain treatments had the highest pentose content, 20.5%; the gum from the papain had the lowest pentose content, 14.4%. The differences between the four gums in pentose content are not great, however, and they can be considered as of the same general order.

Similarly, although the differences between treatments are magnified in the malt gums, owing to higher pentose content, there is considerable similarity between pentose values for all malt gums. Pentose values for malt gums are approximately four times higher than those for the corresponding barley gums.

The barley gum data suggest that the material that is brought into solution during aqueous extraction of untreated barley is similar in composition to the initially soluble gum. Thus, though the effects on yield and viscosity of barley gum suggest that two enzymes are involved in the production of soluble material from insoluble material and in degradation of gum, there is little evidence that the difference between the two enzymes is that one is a hexosanase and the other a pentosanase.

The data for malt gum also indicate that the material brought into solution by enzymatic action during aqueous extraction of untreated malt is similar in composition to the initially soluble malt gum. Again there is little evidence to differentiate between pentosanase and hexosanase activity.

Effect of Malting. Although certain comparisons have been made in previous sections between barley and malt gums, it is advisable to draw these together in order to present some hypotheses on the changes from barley to malt.

Untreated barley contains two components that contribute to the gum obtained on aqueous extraction. One of these is initally soluble in water and one is brought into solution through degradation by enzymes active during extraction. These two materials are similar in composition and the initially insoluble material contributes about 40% to the yield of gum material produced from untreated barley (cf. Table I). Both are degraded to smaller size during aqueous extraction of barley as is indicated by the low solution viscosity of gum from untreated barley.

Malt also contains two components. However, the ratio of these is quite different from that of barley as the initially insoluble material contributes about 80% to the weight of gum obtained from untreated malt (cf. Table I). This suggests that the activity of the gum-degrading enzymes of malt is much greater than that of barley, which is to be expected. The solution viscosities of the gums from alcohol-treated malt are much lower than those of the corresponding barley gums, and this clearly indicates that marked changes in molecular size and other properties of gums have occurred during malting.

Table I shows that the amount of initially insoluble gum – that obtained from the combination of boiling alcohol and papain treatments of grain – decreases from barley to malt. This suggests that degradation of gum takes place during malting and that some initially soluble gum disappears. As yields of gum from the untreated barley or malt are equal, more insoluble material is brought during malting to the stage at which it becomes soluble during aqueous extraction. Perhaps the increase in enzymatic activity in the kilned malt is responsible for this. However, there are changes in composition from barley to malt that must also be considered.

The change in gum composition from barley to malt is indicated in Table IV, which gives the yield of glucose and pentose in gum as percentage of grain. One cannot ascertain whether any of the initially soluble barley gum is carried into the malt, but this is not important as the initially insoluble material is similar in compostion to the ini-

TABLE IV

AMOUNT OF GLUCOSE AND PENTOSE IN GUM AS YIELD FROM GRAIN

Treatment	Glucose		Pentose	
	Barley	Malt	Barley	Malt
	%	%	%	%
None	1.96	0.66	0.42	1.82
Papain	1.52	0.18	0.26	0.47
Alcohol	0.83	0.05	0.17	0.26
Alcohol and papain	1.11	0.06	0.29	0.48

tially soluble gum. The amount of glucose in malt is considerably less than that in barley, but the amount of pentose is increased. Malt gums obtained after alcohol treatment are much less viscous in solution than corresponding barley gums, but it is impossible at this time to decide whether this is the result of a change in composition or because of general degradation to lower molecular size. Both factors are probably involved.

The differences between pentosan and glucosan responses to malting are not in accord with the indicated activities of gum-degrading enzyme systems of barley and malt that operate during aqueous extraction of the gum. Although two enzyme systems appear to be involved, there was no clear-cut evidence to differentiate activities of pentosanase and hexosanase during aqueous extraction. Activity of enzymes during malting thus appears to be different from that occurring during aqueous extraction of barley and kilned malt. This point requires further study to determine whether or not different enzymes are active during malting and during extraction.

There is still considerable uncertainty regarding the nature of barley and malt gums. Preece and his group (16–20) suggest that the main component of barley gum is a polymer of glucose residues linked by β-glycosidic bonds, and that the characteristic change from barley to malt is the disappearance of this β-glucosan. The present studies have also indicated a high proportion of glucose in barley gum and considerably smaller amounts in malt gum. Preece's β-glucosan has also been isolated and used in other studies (2), but chemical combination in the gum between pentose and hexose is still a possibility. The close similarity in composition between gums from raw and enzyme-inactivated barley, and also the similarity between gums from raw and treated malt, suggest more than physical mixtures of hexosan and pentosan.

If the insoluble material is composed of linked pentoses and hexoses, a single endo-glycosidase might reduce the molecular size during malting to produce water-soluble gum of the same composition. Other enzymes active during malting may then operate on the water-soluble material and eventually change its composition.

The amount of glucosan decreases markedly during malting, and the amount of pentosan increases. Thus pentosanases and hexosanases operate at quite different rates during malting, so that the activity of gum-degrading enzymes during the malting process requires study. Enebo, Sandegren, and Ljungdahl (7) suggest that a transglycosidase may operate at this stage, possibly with pentosan units being transferred with subsequent release of hexosan material. This is a reasonably satisfactory working hypothesis, and our results do not contradict it. At least, the pentosan material of barley and malt does not appear to be as readily reduced to simple sugars as the hexosan material. There is even some difference in opinion as to whether malt contains any free pentoses (8, 9, 10, 11, 14, 18), though they are produced, but may disappear again, during the malting process.

In spite of considerable study of barley and malt gums and of the enzymes that degrade them, a close relation between these and malting behavior has yet to be demonstrated. There is actually good reason for considering that such a relation exists, and that it should be clearly demonstrable. The isolation and analysis of barley and malt gums (12, 13, 20) and the use of several types of barley gum (2, 3, 4, 19) and of substituted cellulose (7, 22) as substrates for detection and measurement of cytolytic activity have provided much useful information and have opened up a neglected field of research. The information has provided bases for hypotheses on malting behavior, and the search for another missing factor in barley evaluation appears to be narrowing down. Study of green malt at various stages of growth, by new technics perhaps, and comparison of the enzyme systems with those of barley and kilned malt, should materially clarify the situation. Development of tests for evaluation of barleys and malt may thus be put on a firmer base.

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PRECIPITATION BY VARIOUS SALTS OF THE PROTEINS EXTRACTED BY FORMIC ACID FROM WHEAT, BARLEY, RYE, AND OAT FLOURS[1]

D. K. CUNNINGHAM, W. F. GEDDES, AND J. A. ANDERSON

ABSTRACT

The precipitation by salts, of proteins extracted with 0.01 *N* formic acid from wheat, barley, rye, and oat flours, has been studied. When salt concentration was expressed as ionic strength, sodium formate was more effective than sodium chloride for all proteins. Barley protein was most easily precipitated by sodium or calcium formates or chlorides, rye protein least easily, and wheat protein intermediate; oat protein varied in comparative behavior with the salt employed. Calcium and barium chlorides were less effective than sodium or potassium chlorides when tested with wheat protein. Decreasing the hydrogen-ion concentration increased the effectiveness of sodium formate as a precipitant for wheat, barley, and oat proteins.

The preparation of a series of analogous proteins from wheat, barley, rye, and oat flours, and studies of certain of their physical and chemical properties, have been described in an earlier paper (2). This study has been extended by investigation of the precipitation of the proteins by various salts from 0.01 *N* formic acid solution and from this solution after stepwise decreases in the hydrogen-ion concentration. There is an extensive literature on the salting-out of protein, but for present purposes it seems adequate to cite only the able review by Cohn and Edsall (1).

Materials and Methods

Methods of Precipitation. Protein solutions used in these studies were prepared from the series 2 flours described in (2) with protein content: wheat 11.0%; barley, 11.1%; rye, 8.9%; and oat, 11.2%, on an N $\times$ 5.7 dry-matter basis. Proteins were extracted from 20 g. of the flours with 400 ml. of 0.01 *N* formic acid; precipitation studies were made on 50 ml. aliquots of the protein extracts in 100 ml. centrifuge tubes. The amount of salt solution required to give the desired ionic strength was added and the solution was stirred for 1 minute. Tubes were allowed to stand for 10 minutes before centrifuging, and an aliquot of the supernatant solution was then taken for determination

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of protein. For the first few experiments, the solid precipitate was washed out of the tube into a second Kjeldahl flask. The tube was then swabbed out with filter paper, which was added to the flask, and nitrogen was determined. The sum of peptizable nitrogen plus precipitated nitrogen was then compared with the total nitrogen of an untreated sample. In all cases the agreement was close; accordingly, in the later tests only the supernatant solution was analyzed, and the nitrogen in the precipitate was calculated by difference.

Adjustment of pH Levels. Initial comparisons of the effectiveness of various precipitating salts were made with the original formic acid extracts of pH 3.0–3.2. As a next step, precipitations were studied at higher pH levels.

The pH was raised either by dialyzing the acid solution in Visking tubing against flowing distilled water, or by treating the protein solution directly with ion-exchange resins. The dialysis method met with only limited success since degradative changes similar to those described by Olcott, Sapirstein, and Blish (5) were noted in the dialyzed solution.

The Amberlites IRA-400 and IR-100H, strong anion and cation exchange resins respectively, have been used in mixture by Reid and Jones (6) to alter the ionic strength of plasmas without altering the pH. By suitable changes in the ratio of one to another, the pH of protein solutions could be raised while reducing the ionic strength so that no precipitation of proteins took place.

The protein solution (400 ml. containing 1.6 g. of protein) was stirred with 20 g. of a mixture of five parts Amberlite IRA-400 anion-exchange resin and one part Amberlite IRA-120 cation-exchange resin, while observing the pH on a continuous reading electrometric titrimeter. When the desired hydrogen-ion concentration was reached, the mixture was immediately poured off through a linen disk in a Büchner funnel, and centrifuged to remove suspended solids. Aliquots were used for salt precipitation studies.

This treatment caused a loss of about 10% of the nitrogen for the anion-exchange resins; no loss was noted for the cation-exchange resin. It was assumed that this loss to the anion-exchange resin was distributed equally over the gluten constituents and so did not alter the properties of the gluten. This assumption was partly justified for wheat gluten by comparison of gluten precipitated from such a solution to hand-washed gluten and to gluten precipitated by saturated calcium hydroxide from untreated solution. No significant differences in elasticity or cohesiveness could be observed among the three products.

The proteins reacted differently to changes in hydrogen-ion concentration. The pH of wheat protein solutions could be increased to 7 and higher; barley, and oat protein solutions could not be raised above pH 6.0 without precipitation; rye protein solution treated with anion-exchange resin behaved erratically, and reproducible results could not be obtained for this cereal at pH levels higher than 3.2.

Results

Precipitation or solubility data for proteins are recorded by plotting percentage of total protein precipitated or solubilized against some function of the concentration of precipitating agent. For dissociable salts, following the work of Mellanby (4) and of Lewis and Randall (3), the concentration function usually employed has been the ionic strength. Weight as a concentration function is meaningless except for empirical studies; molarity compares salts on a numerical basis, but does not take into account the valence of the ions. Ionic strength by definition includes both the number and the valence; comparisons made on this basis indicate more subtle differences resulting from individual properties of the ions such as ionic radii and mean effective diameter.

Figure 1 compares the precipitation of wheat gluten plotted against

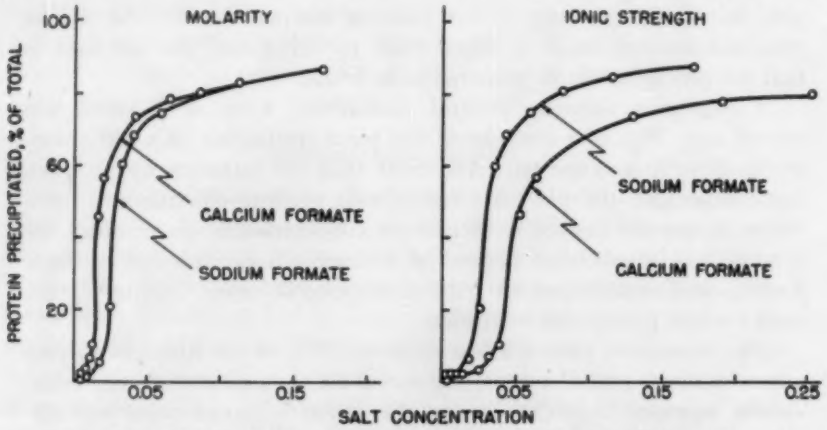

Fig. 1. Precipitation of wheat gluten from 0.01 N formic acid solution with varying amounts of sodium and calcium formates; salt concentration expressed in molarity and ionic strength.

molarity and ionic strength of sodium formate and calcium formate. These curves show that on the basis of molar concentration calcium formate is the more efficient precipitant, but on the basis of ionic strength sodium formate is the more efficient precipitant. Curves similar to Fig. 1 have been prepared for the other cereal proteins. At

the same ionic strength sodium formate is always a more effective precipitant than calcium formate. Since it was of interest to compare the various salts independently of their valences, all further comparisons were made in terms of ionic strength.

Comparison of Sodium Formate and Sodium Chloride. Figure 2

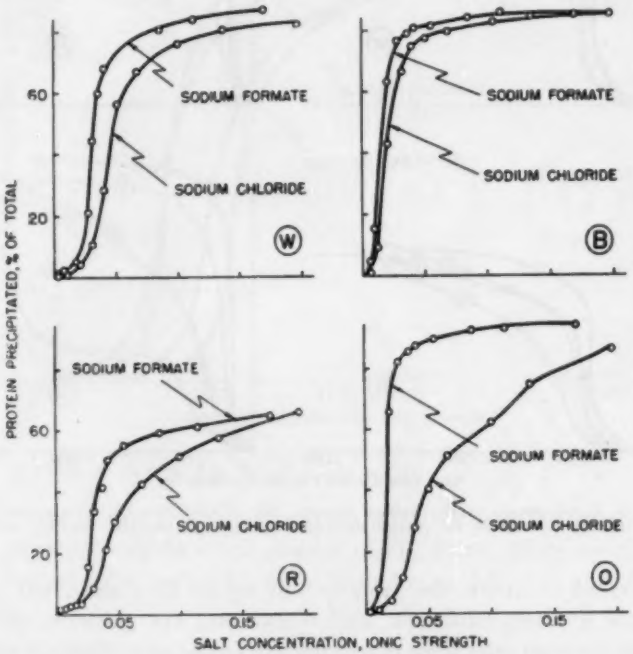

Fig. 2. A comparison of sodium chloride and formate in the precipitation of (W) wheat, (B) barley, (R) rye, and (O) oat protein dissolved in 0.01 N formic acid.

compares the effects of precipitation by sodium formate and sodium chloride of the proteins of wheat (W), barley (B), rye (R), and oats (O). The chloride ion is a less effective precipitant than the formate ion. The difference between the ions is least for barley and very marked for oats, whereas wheat and rye show intermediate differences. Rye also differs from the other cereals in that a smaller percentage of the total protein is precipitated.

Comparison of Sodium and Calcium Formates and Chlorides. The effect of four salts, sodium formate (A), calcium formate (B), sodium chloride (C), and calcium chloride (D) in the precipitation of the cereal proteins from 0.01 *N* formic acid is shown in Fig. 3 for wheat (W), barley (B), rye (R), and oats (O). Barley protein appears to be

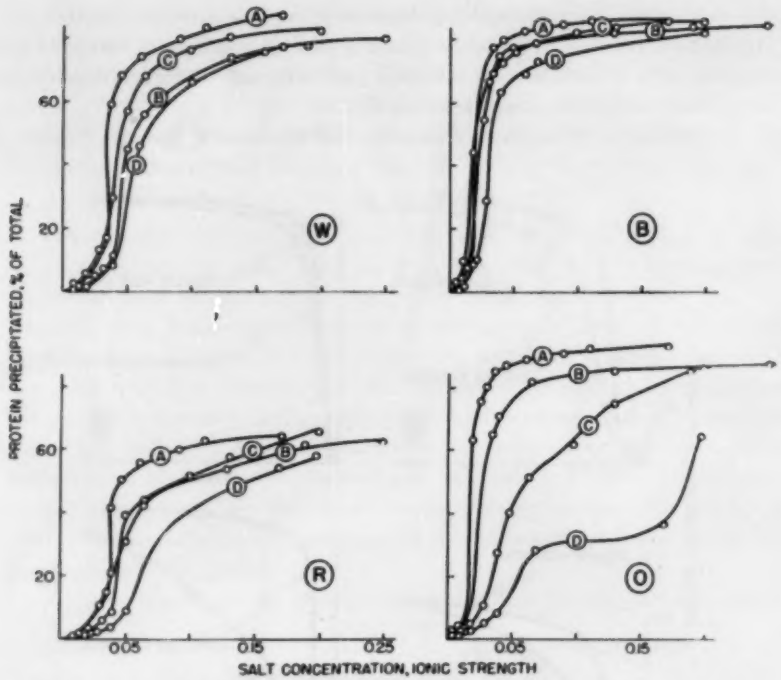

Fig. 3. A comparison of (A) sodium formate, (B) sodium chloride, (C) calcium formate, and (D) calcium chloride in the precipitation of wheat (W), barley (B), rye (R), and oat (O) proteins.

precipitated to about the same extent by all four salts; oat protein gives the greatest variation, and wheat and rye proteins are intermediate. Sodium salts were more effective in the precipitation of wheat protein than calcium salts; for barley and rye proteins, the differences were not significant, and for oat protein, calcium formate was more effective than sodium chloride.

Comparison of Sodium, Potassium, Barium, and Calcium Chlorides. Figure 4 compares the effects of precipitation of wheat protein in 0.01 *N* formic acid with sodium, potassium, calcium, and barium chlorides. These results confirm those of Fig. 3, in that sodium and potassium chlorides are more effective than calcium and barium chlorides.

Precipitation at Different pH Levels. Results are shown in Fig. 5 for precipitation of wheat protein (W) at three pH levels and of barley protein (B) and oat protein (O) at two pH levels. To compress the scale of salt concentration, protein precipitated is plotted against the logarithm of ionic strength. The curves indicate that the sensitivity to

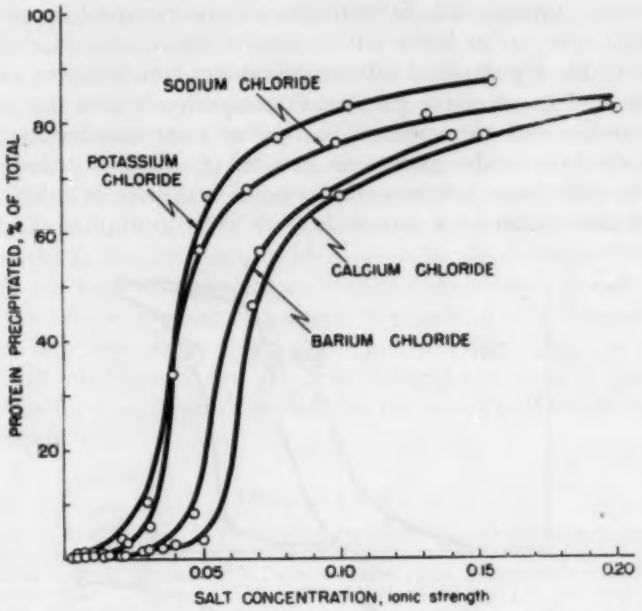

Fig. 4. A comparison of sodium, potassium, calcium, and barium chlorides in the precipitation of wheat gluten from 0.01 N formic acid solution.

salt increased as the hydrogen-ion concentration was decreased. This is most clearly shown by wheat gluten, in Fig. 5 (W). Here solutions in 0.01 *N* formic acid (pH 3.1) were brought to pH 5.0 and 6.0 respectively.

General Discussion

The investigations showed that the proteins of wheat, barley, rye, and oats, in dilute formic acid, may be easily precipitated by small additions of neutral salts; that the salts vary in effectiveness with both the cation and the anion employed; and that a large increase in the effectiveness of the salts may be produced by decreasing the hydrogen-ion concentration of the protein solution. The most probable explanation of this behavior of the salts towards the solubilized proteins may be the formation of insoluble protein salts.

This hypothesis is suggested by the ease of precipitation of the proteins by small quantities of salts, and by the antagonism between hydrogen-ion and sodium formate shown in the experiments on altering the pH level. In solutions of greater hydrogen-ion activity large amounts of cation are required to convert the protein-hydrogen-ion

complex to a protein salt. As hydrogen-ions are removed by solution, precipitation occurs at lower salt concentration.

This simple hypothesis of salt formation does not, however, explain the sigmoidal shape of the precipitation curves, whereby the protein precipitated is not proportional to the salt concentration but rises rapidly after the initial increments of salt are added; nor does it explain the differences between the formates and salts of other acids. Some of these differences may arise from the constitution of gluten,

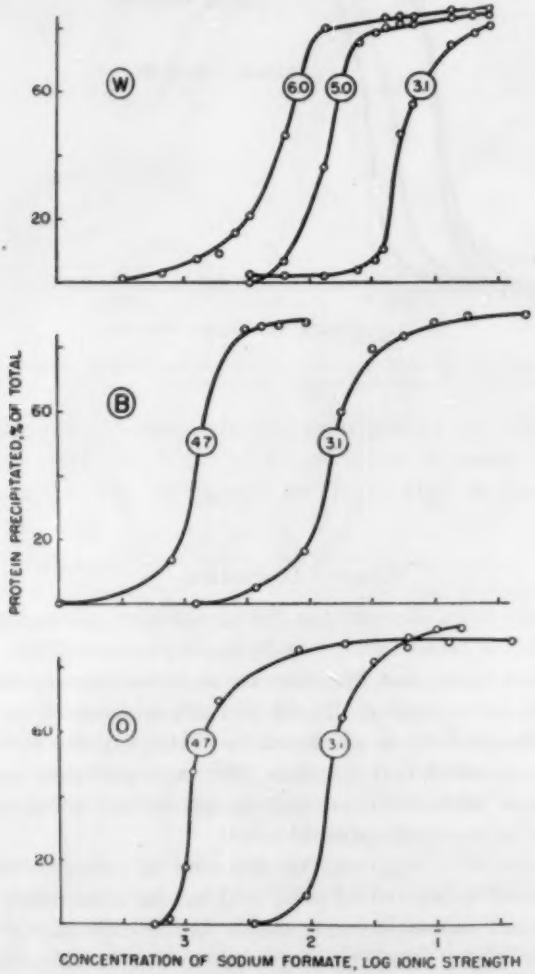

Fig. 5. Precipitation of (W) wheat, (B) barley, and (O) oat protein from formic acid solution at varying pH levels.

which appears to be a complex of closely related proteins rather than a homogeneous material.

These experiments have shown that the proteins of wheat, barley, and rye, and to a lesser extent, of oats, are precipitated in a similar way by neutral salts from formic acid solution. While a study of the action of salts on the pure proteins would have been preferable, the insolubility of the cereal proteins necessitated the use of dilute formic acid. However, an indication of the probable behavior of the proteins towards salts in the absence of acid is given by the studies at reduced hydrogen-ion concentration. These studies indicate that the effectiveness of the salts as precipitating agents is enhanced by the removal of hydrogen-ion; the very pronounced precipitating effect of small amounts of salt suggests that salt-protein complexes may be partially responsible for the known insolubility of the proteins of wheat, barley, rye, and oats.

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A RECORDING GEL TESTER[1]

E. T. HJERMSTAD[2]

ABSTRACT

The dynamometer-recorder mechanism of the Corn Industries Viscometer was adapted to measure the force required to pull an embedded disk from a gel. Chart curves defining the stress-strain relationships and yield points of gels were automatically obtained.

The measurements of the gel properties of materials such as starches, pectins, and gelatin are at present made by a number of different instruments and procedures. All of the methods commonly used require close attention, skilled manipulation, and consistent interpretation of values obtained. Many of the commonly used methods are apt to give results which reflect idiosyncrasies in the technic of the operator. Some of the gel testers in use determine only the breaking strength of gels, while others give a measure of the deformation under applied stress.

A common failing of several gel testers is the difficulty involved in achieving uniform, reproducible rates of applying force to the gels. Another is the necessity of increasing the applied force intermittently. This is apt to result in erroneous yield-point values.

The experimental gel tester was designed with the object of securing a completely automatic method of measuring the deformation under increasing load and the yield point of gels. It is so constructed that chart records of these properties are simultaneously registered.

Principle of the Method

The gel tester is designed to utilize the embedded disk method of measuring gel strength which was first proposed by Saare and Martens (9) and later modified by Kerr (4), Hamer (3), and Bechtel (2). These investigators have shown that a relatively high degree of sensitivity and precision can be obtained by the use of an embedded disk for the measurement of rigidity and breaking strength of gels.

The system which was devised for withdrawing the embedded disk is of extreme simplicity. It is essentially a device which lowers at a slow uniform rate a gel containing an embedded disk which is attached by a cord directly to a dynamometer capable of registering the desired range of force. As the gel is slowly lowered the dynamometer reacts upward with a continuously increasing force on the disk until

[1] Manuscript received December 23, 1952.
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the yield point of the gel is reached. The essential mechanical principles involved are illustrated in Fig. 1. This method of exerting force

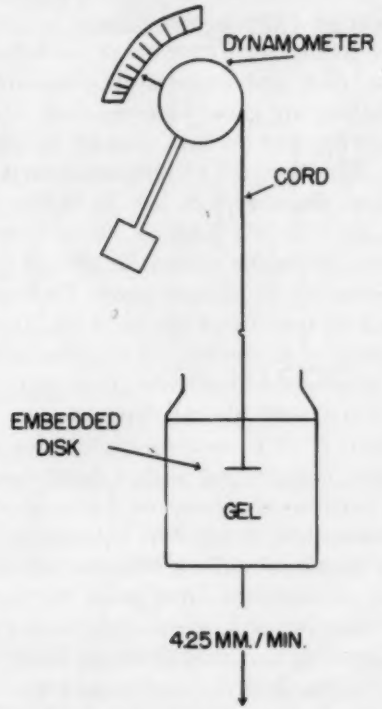

Fig. 1. Mechanical principle used for gel testing.

on the embedded disk appears to offer the following practical advantages:

1. The force is increased continuously rather than intermittently. 2. Sources of mechanical friction are minimized. 3. The motion of the dynamometer can easily be transmitted to a chart-recorder mechanism to produce a continuous curve defining the applied force, the deformation of the gel, and the yield point of the gel. 4. By suitable loading of the weight arm this type of dynamometer can be adapted to measure rapidly a relatively wide range of force. By proper magnification of the displacement, small force intervals are distinguishable. 5. The method is adapted to completely automatic operation.

Experimental Apparatus Used

The gel-lowering device consists of a shaft mounted vertically in a stationary sleeve allowing free motion up and down and supported by

a rack-and-pinion gear connected through a speed reducer to a constant-speed electric motor. The jar containing the gel is held level by a clamp at the top of the shaft. By use of extreme gear ratios a uniform downward movement of 4.25 mm. per minute is obtained.

Force is applied to the gel by means of an embedded disk. Construction of the jar, disk, and cover is approximately that proposed by Bechtel (2). The jars are glass, wide-mouthed, of 150-ml. capacity, 59 mm. inside diameter, and 85 mm. over-all height. The disks are copper, 19 mm. in diameter and 1.5 mm. thick, attached centrally to a rigid rod of 2.2 mm. diameter with a hook on the other end. A flat washer is fixed on the rod in a position which allows the disk to be supported level 3 cm. below the surface of the gel when the disk rod is placed in the center of the slotted cover. For convenience a horizontal line is etched on the side of the jar 3 cm. above the supported disk. Gels are prepared up to the level of this line in the jar. The disk-rods are adjusted to have the same over-all weight.

In this experimental assembly the dynamometer system of a Corn Industries Viscometer (1, 5) is used to apply force to the embedded disk. This viscometer is equipped with a pendulum-arm type dynamometer which is capable of measuring forces up to 1000 g. with a sensitivity of approximately ± 2 g. The dynamometer is attached to an Esterline-Angus continuous chart recorder which records applied force versus time as a continuous curve. Since the force resulting from the torque at the cable drum is transmitted to the dynamometer by means of a cord extending horizontally to the cable drum, it was convenient to by-pass the cable drum and extend this cord out beyond the viscometer, over a low-friction pulley, and down to the hook of the embedded disk in the gel-testing assembly, as in Fig. 2. As the jar is moved downward, force is applied to the gel by the dynamometer arm and a curve is drawn on the recorder chart. This curve defines both the force applied and the deformation of the gel relative to time. When the yield point of the gel is reached, a sharp leveling of the curve is recorded, as in Fig. 3.

The dynamometer in the Corn Industries Viscometer is ordinarily adjusted to register a zero reading on the chart when the viscometer propeller is running in water. Therefore it is necessary to attach a counterweight to the cord running down to the disk rod. The combined weight of counterweight and disk rod should produce a zero reading on the chart.

For convenience, the cord leading to the gel disk may be attached to the regular viscometer cord at point *A* as in Fig. 2. When the dynamometer-recorder assembly is being used for gel-testing, the sec-

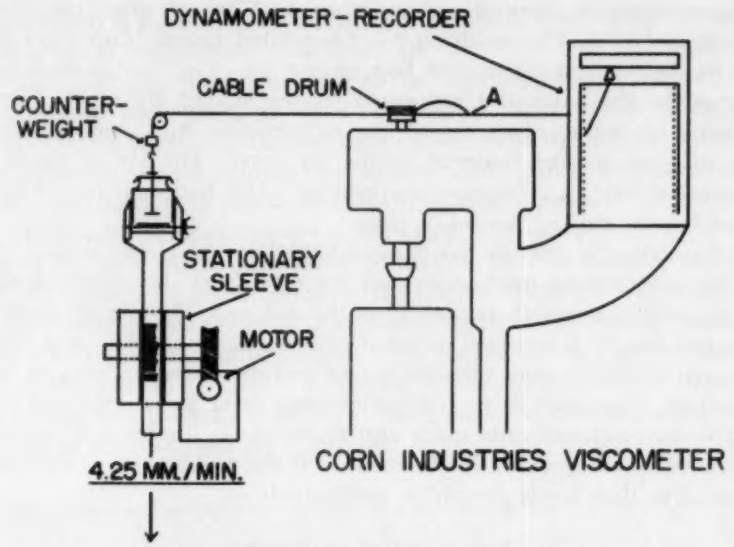

Fig. 2. Schematic diagram of gel testing assembly.

tion of cord between point *A* and the cable drum will become slack and will not interfere with the motion of the cord to the gel-disk. When the viscometer is being used for viscosity determinations the section of cord from point *A* to the gel-lowering assembly may be made slack by supporting the counterweight.

The chart which is at present provided with the Corn Industries Viscometer is scaled in gram-centimeters of torque. To convert to force expressed in grams it is necessary to divide the gram-cm. reading by the effective radius, in centimeters, of the cable drum. This radius is 1.78 cm. However, it was found to be convenient to attach to the end of the dynamometer arm a supplementary weight capable of adjusting the displacement of the arm to the correct scale reading expressed in grams. Other supplementary weights of increasing magnitude can be attached, so that scale readings of ½, ¼, or ⅛ of the applied load in grams will be obtained.

Gel-Testing Procedure

Since the properties of gels, especially those of starch gels, are greatly influenced by the method used in preparing the pastes, it is essential that uniform, readily reproducible cooking conditions be used. It was found convenient to use the Corn Industries Viscometer for the preparation of starch pastes. The starch is cooked in the vis-

cometer using the desired concentration, bath temperature, propeller speed, and time. The resulting paste is poured rapidly into a gel jar up to the level of the etched line on the jar. The disk is then immersed in the paste and supported by the slotted jar cover. Light mineral oil is poured on the surface to minimize skin formation and the disk-rod is then centered in the jar cover. The jar of paste is supported level in a constant-temperature water bath and cooled and aged for the desired period of time.

To make the test the jar is placed level in the clamp at the top of the gel-lowering mechanism and the cord from the dynamometer is attached to the hook at the end of the disk-rod. The proper supplementary weight is attached to the dynamometer arm. The jar is then lowered manually until the cord is taut and the chart-pen rests on the zero line. The chart drive and gel-lowering drive are started and the test is then automatically made and recorded. As the force is applied a gradually rising curve is obtained until the yield point is reached. The curve then levels sharply or suddenly drops.

Interpretation of Results

The load in gram-centimeters can be read directly from the chart at any point on the curve or this value may be converted to grams as indicated above.

The gel deformation at any point can also be determined solely from the chart curve, since a practically constant ratio exists between the distance the disk moves downward and the distance the pen moves up on the chart. The deformation represented by a given scale reading on the chart may be found by subtracting the distance the disk moves downward from the distance the jar moves downward during a given time interval. The distance the disk moves downward to produce any given scale reading on the chart may be easily determined by tying the disk to the jar and running the jar-lowering mechanism until a curve is drawn across the chart. Since the disk is tied to the jar, the distance the disk moves is equal to jar speed multiplied by time. Chart scale readings at given elapsed time periods can then be plotted against millimeters of distance the disk moves downward and a calibration curve can be drawn using rectangular coordinates. The millimeters deformation at any given scale-reading on a chart curve for a gel test can then be determined by subtracting the distance the disk moved to give this scale reading (obtained from calibration curve) from the millimeters of distance the jar moved (jar speed $\times$ elapsed time). For convenience, a conversion table may be set up giving the

millimeters deformation represented by any load reading on the present chart at any elapsed time interval.

The data obtained from the curve can therefore be plotted on rectangular coordinates to show the load vs. deformation characteristics of gels. However, the recorded curves show the relative rates of deformation and for routine comparisons made, using identical instrument constants, such conversion should not be necessary.

The yield point of the gel is indicated by a sharp leveling of the curve. In some of the gels tested no further increase in force was obtained, though no visible break in the surface of the gel was apparent at the yield point. Other gels appeared to yield at a fairly constant force in replicate determinations, then higher forces were registered until the gels finally broke, as shown in Fig. 3. The higher strength indicated just prior to breaking is believed to be due to surface-skin effects at the oil-starch gel interface.

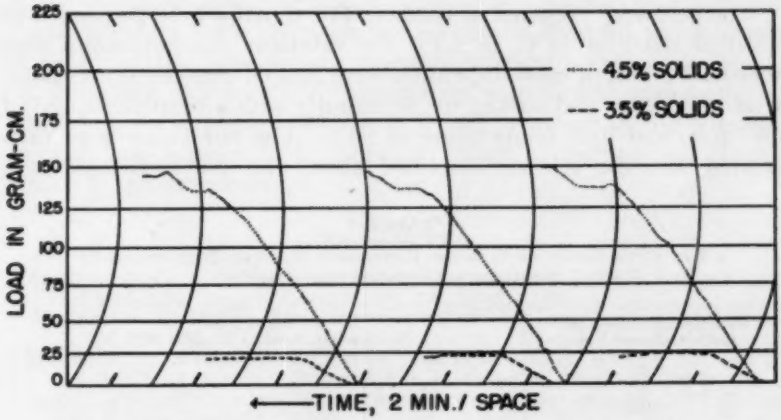

Fig. 3. Chart curves of replicate unmodified corn starch gels drawn by recorder mechanism of Corn Industries Viscometer.

Sensitivity and Reproducibility

The experimental gel tester is capable of measuring a very wide range of gel-strength. Gels made from corn starch in a concentration of 3.5% dry substance produced curves with clearly indicated yield points, as shown in Fig. 3. The reproducibility of the test is chiefly dependent on the uniformity of the procedure used in preparing the dispersions from which the gels are formed. The average variation obtained in tests of replicate gels with the experimental tester did not exceed $\pm 5\%$ of the mean load at the yield point.

Gel Measurements of Starches Containing Low Proportions of Hydroxyethyl Ether Groups

The substitution of low proportions of hydroxyalkyl groups in gelling starches such as corn and wheat starch by etherification reaction results in substantial lowering of their gelling and retrograding tendencies (6, 7, 8). For the purpose of demonstrating the sensitivity of the experimental gel tester a number of gel-yield-point determinations were made on gels of starches containing very low proportions of hydroxyethyl ether groups.

The starches were prepared in the laboratory from commercial unmodified corn starch and contained proportions of hydroxyethyl groups ranging from 0.002 to 0.043 per anhydroglucose unit. All of the starches exhibited paste viscosities equivalent to that of unmodified corn starch when subjected to standard alkali fluidity tests and after cooking for 30 minutes in the Corn Industries Viscometer with a bath temperature of 98°C.

The gels were prepared as follows: The starches were suspended in distilled water at 25°C. in 5.4% dry substance concentrations. One thousand grams of each suspension were poured into the Corn Industries Viscometer and cooked for 30 minutes with a propeller speed of 60 r.p.m. and bath temperature of 98°C. The hot pastes were then poured into the gel jars described above and the disks and cover

TABLE I

GEL YIELD POINTS OF STARCHES CONTAINING VARYING PROPORTIONS OF SUBSTITUTED HYDROXYETHYL GROUPS

Hydroxyethyl Group per Anhydroglucose Unit	Gel Yield Point (5.4% solids, Aged 24 Hours at 20°C.)	
avg.	*g.*	*avg.*
None	137 127	132.0
0.002	123 120	121.5
0.005	105 100	102.5
0.011	90 85	87.5
0.021	58 56	57.0
0.032	37 36	36.5
0.043	19 17	18.0

assemblies put in place. The gels were aged for 24 hours in a water bath maintained at a constant temperature of 20°C. Duplicate gels were prepared.

The aged gels were placed in the experimental gel tester and yield point determinations were made according to the procedure described above. The results are summarized in Table I.

The data in Table I illustrate the sensitivity of the experimental gel tester. Starch containing an average substitution of one hydroxyethyl group per 500 anhydroglucose units showed a gel yield point significantly lower than that of the control sample. Increasing proportions of substituted hydroxyethyl groups resulted in smoothly decreasing gel yield point values obtained with this gel tester.

Discussion

The experimental assembly described above was designed to utilize the dynamometer-recorder system of the Corn Industries Viscometer. However, the sensitivity and load range of the dynamometer arm and the scale values of the chart are not necessarily ideal for measurement of gel properties. The present assembly provides a convenient means of obtaining reproducible, automatically recorded measurements which are not dependent on the skill and judgment of the operator. It, therefore, appears to be especially suited to routine and control testing of gel properties.

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THE EFFECT OF THREE LEAF AND STEM RUST CHEMOTHERAPEUTANTS ON THE BAKING BEHAVIOR OF WHEAT[1]

PAUL J. MATTERN AND J. E. LIVINGSTON[2]

ABSTRACT

Mixing and baking tests were conducted on hard red winter wheat which had been treated during the growing season with calcium sulfamate, Actidione, and sodium sulfanilate. Calcium sulfamate induced variations in the properties of the harvested seed which were detrimental to baking properties.

Many useful chemical sprays may be developed as fungicides which either become systemic in the plant or induce over-all changes in the physiology and thereby alter the reaction of the plant to disease (1, 3, 4). As the term would imply, systemic fungicides are absorbed and transported through the plant tissues. For this reason it is logical to question whether or not these foreign substances introduced through the leaves during the growth of the plant will produce variations in the properties of the harvested seed. This paper reports an investigation of the baking properties of flour milled from Pawnee winter wheat which had been treated during the growing season with calcium sulfamate, Actidione,[3] and sodium sulfanilate. These treatments were employed because the fungicides proved to be the most promising of a large number tested by Livingston (3).

Materials and Methods

The first applications of the systemic fungicides were made during the flowering period. Second applications were given 10 to 14 days later. Details of the spraying technics have been presented by Livingston (3).

The wheat samples are identified as follows: Sample A: No chemotherapeutic treatment. Sample B: One application of calcium sulfamate, 9 lb. per acre. Sample C: Two applications of Actidione (2), 9 g. per acre each application. Sample D: Two applications of sodium sulfanilate, 9 lb. per acre each application.

The samples were milled on an Allis-Chalmers experimental mill to an 85% patent flour. A National Micro Recording Mixer was employed in obtaining the mixing curves (5, 6). A 30-g. sample of flour,

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[3] An antibiotic substance isolated form the beers of streptomycin-producing strains of Streptomyces griseus.

on a 14% moisture basis, with normal baking absorption, was used for each curve. The flour protein levels on the four samples were within a range of 13.9–14.4%, and averaged 14.2%.

The micro baking technic described by Van Scoyk (7) was used with the A.A.C.C. basic formula with the sugar at 6%. Because the samples required no oxidation, only the nonoxidized bake is described. Doughs were proofed to a constant height of 6.3 cm. in pans scaled down from the standard high-form pans.

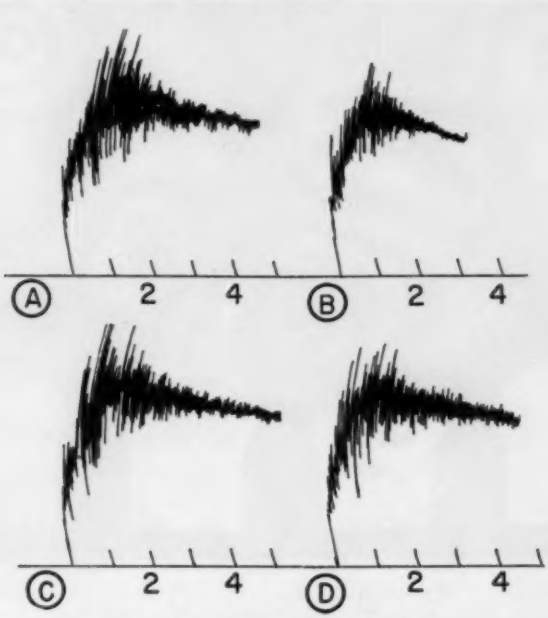

Fig. 1. Recording-micro-mixer curves of flour from wheat treated with systemic fungicides: A, no treatment; B, calcium sulfamate; C, Actidione; D, sodium sulfanilate.

Results

Representative mixing curves are shown in Fig. 1. The curve from Sample B, which had been treated with calcium sulfamate, exhibited a short mixing time and rapid breakdown of the dough in comparison with the control. This curve is typical of those produced when certain reducing agents are added directly to the dough.

To check the effect of adding this fungicide directly to the flour, the control flour, with 6 mg. % of calcium sulfamate added, was run on the mixograph. However, the mixing curve was little changed from that of the control. Therefore it appears that the deviation of

curve B is not due to residual calcium sulfamate in the flour, but to deleterious changes that took place in the wheat during the growing season.

Samples C and D, treated with Actidione and sodium sulfanilate, respectively, produced curves closely paralleling that of the check. The normal dough development of these two samples was not impaired by their chemical treatment.

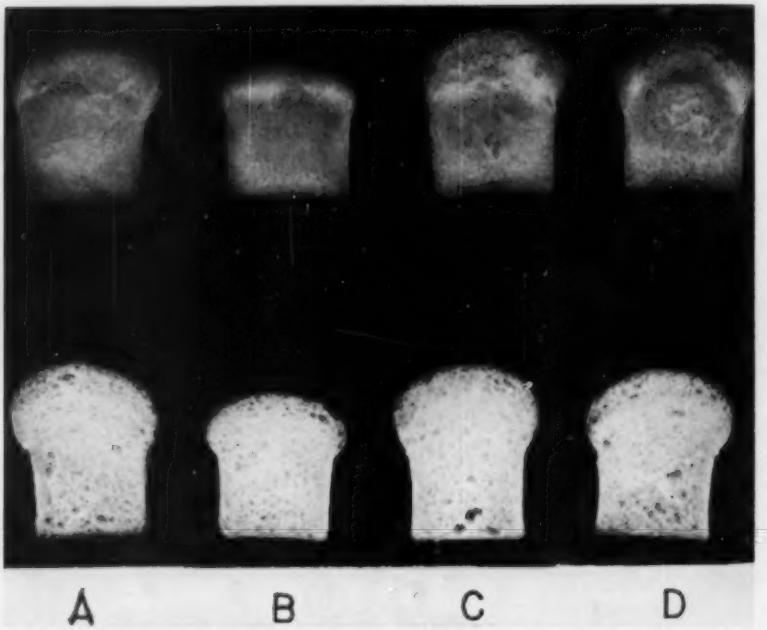

Fig. 2. Loaves baked by the basic procedure using flour from wheat treated with systemic fungicides: A, no treatment; B, treated with calcium sulfamate; C, treated with Actidione; D, treated with sodium sulfanilate.

Figure 2 shows bread baked from the four samples used in this study.

	Proof Time *min.*	*Volume* *cc.*
Sample A, no treatment	53	169
Sample B, calcium sulfamate	49	137
Sample C, Actidione	55	173
Sample D, sodium sulfanilate	56	157

Again, sample B illustrates deleterious effects resulting from the use of calcium sulfamate. The loaf is small as compared with the check and there is a tendency towards a more open grain.

Rebaking sample B with 1 mg.% and 2 mg.% levels of potassium

bromate produced a slight improvement in grain and texture, but the volumes remained unchanged. Baking the control flour with 6 mg.% of calcium sulfamate added did not change normal baking characteristics.

The calcium sulfamate bake from 1953 reported herein corroborates the findings from the 1952 baking tests in which Cheyenne wheat was used. The detrimental factor must be induced from the sulfamate group since sodium sulfamate also produced wheat having poor baking quality during both years of testing.

Flour from samples C and D, treated with Actidione and sodium sulfanilate respectively, produced loaves which compared favorably with the check, further substantiating the findings on the mixing curves.

Discussion

Actidione and sodium sulfanilate were effective in the control of rust, and produced wheats which performed satisfactorily in the baking test. Sodium sulfanilate, which heretofore has not been reported as a systemic fungicide, appears to warrant further investigation. Calcium sulfamate gave excellent control of leaf and stem rust in the field, but produced deleterious changes in the wheat when evaluated by the baking test, illustrating that chemotherapeutants sprayed on growing wheat, and acting as systemic fungicides, may cause basic changes in the wheat seed during the growing season.

Chemotherapeutants which give good fungicidal control in the field, and do not impair the baking performance, should be subjected to toxicological tests. If one or more of these chemotherapeutants should prove to be nontoxic, such differences as costs and range of safety between fungicidal effectiveness and phytotoxicity must be considered before they can be recommended for practical use.

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A STUDY OF INTERNAL MOISTURE MOVEMENT IN THE DRYING OF THE WHEAT KERNEL[1]

H. A. Becker and H. R. Sallans[2]

ABSTRACT

The standard solution of the nonstationary-state diffusion equation for diffusion out of spheres is applicable to the drying of wheat kernels, the diffusion coefficient being independent of moisture content in the practically important drying range 12–30%. A method for evaluating the coefficient is described; numerical values for the coefficient were found to lie between 0.069×10^{-6} and 2.77×10^{-6} cm^{2}sec^{-1} in the temperature range 20° to 80°C. An Arrhenius type temperature dependency is shown. Energies of activation of 12,920, 14,600, and 16,300 cal/mol were calculated for three different sets of data.

The drying of wheat is a problem of considerable practical importance and has been extensively studied. A large number of investigations have had as their object the establishment of safe limits of operating conditions for the type of dryer studied, i.e., the limits within which the baking quality of the wheat is not damaged. Probably the most comprehensive investigation has been that of Stansfield and Cook (9). This work defined safe operating conditions for the type of dryer used in commercial elevators and still forms the basis for official regulations governing wheat drying in Canada.

Wheat drying has also been studied purely as a drying operation. A number of workers have developed empirical correlations for the prediction of drying rates. A recent monograph (2) sponsored by the American Association of Cereal Chemists gives an extensive review of the present status of research on the drying of wheat. In this work it is stated that "The key problem obviously relates to the simplest case in which each kernel is fully exposed to air of constant temperature and humidity. Until this problem is solved—that is until an equation has been developed . . . that takes account of all the variables involved—bulk grain drying must remain an essentially empirical operation."

A knowledge of the relation among the variables which control the drying of singly exposed kernels appears to be of fundamental importance. The present study was undertaken with the object of formulating a more exact correlation of these variables. Such results should be of value in the design and evaluation of commercial wheat drying equipment.

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The Diffusion Equation

The range of moisture contents most important in practical wheat drying is from 14.5 to 25%, wet basis (17.0–33.3%, dry basis). It is generally agreed that at these moisture levels the rate of internal liquid flow controls the drying rate and that the mechanism of liquid flow is a diffusion mechanism (3, 4, 8). Capillary forces appear to play little part in the flow (8). Under these circumstances it should be possible to correlate drying rates by use of an appropriate diffusion equation. Numerous investigators have utilized approximation formulations of standard diffusion equations (2). It would appear, however, that the following form of the equation for diffusion out of spheres

$$\frac{\bar{m} - m_s}{m_o - m_s} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \quad \exp\ -n^2\pi^2 D_c\, t/r_o^2$$

is best suited to studies on the drying of wheat, since in treating this problem wheat kernels are best described as spherical particles. This equation differs from the usual form in that concentration is expressed as moisture content, dry basis. The parameters are defined as:

$\bar{m}$ = final average moisture content, %;
m_o = uniform initial moisture content, %;
m_s = surface moisture content, %;
D_c = diffusion coefficient, cm^2sec^{-1};
r_o = kernel radius, cm; and
t = drying time, sec.

The derivation of the equation is dealt with in textbooks on diffusion and heat conduction (5, 7).

Babbitt (3), using a differently arranged formulation, was the first to apply the equation to the drying of wheat. His work was done at moisture levels in the range 0–16% and he concluded that in this range the diffusion coefficient decreased with falling moisture content. The equation cannot, however, be rigidly applied unless it is known that the diffusion coefficient is independent of moisture content. Furthermore, unless a method of evaluating the surface moisture level is available the equation must be solved by an uncertain process of trial and error. Some way of overcoming these difficulties is therefore necessary if the correlation is to have satisfactory precision.

It has been noted by Jones (4) that, for a given drying temperature, loss in moisture of wheat in a short drying cycle of fixed duration is a linear function of initial moisture content in the range of moisture

contents 14–30%, dry basis. Furthermore, when the lines for different temperatures are extrapolated they converge on a common intercept on the abscissa in the region of 9.1–10.3% initial moisture content. This phenomenon is also illustrated by the data given in Fig. 5. It was suggested by Jones that this intercept represents the upper level of "more firmly held" water and that when the total moisture content drops below this value the rate of drying falls off very markedly. It appears logical, however, that, aside from any indication of degrees of tenacity of water binding, the intercept must correspond with the effective surface moisture content. If we designate the first term of the diffusion equation as $\overline{M}$, the following rearrangement can be derived:

$$\begin{aligned} \overline{M} &= \frac{\overline{m} - m_s}{m_o - m_s} \\ &= \frac{m_o - m_s}{m_o - m_s} - \frac{m_o - \overline{m}}{m_o - m_s} \\ &= 1 - \frac{m_o - \overline{m}}{m_o - m_s} \end{aligned}$$

The group $\overline{M}$ is therefore equal to unity minus the slope of the regression of moisture loss for unit time on initial moisture content (see Fig. 5). Inspection of the diffusion equation shows that if, for a given temperature, $\overline{M}$ is constant for constant r_o and t, then D_c must also be constant. Jones' study therefore indicates that in the practically important range of moisture contents the diffusion coefficient is, for a given temperature, independent of moisture content. Furthermore, it indicates a method for the evaluation of the surface moisture level. The diffusion coefficient is thus easily evaluated from data obtained by suitably devised methods.

The Fourier series on the right-hand side of the equation can be evaluated for each individual case, but for any degree of accuracy a large number of terms are involved. If a number of solutions are required it is more convenient to prepare a plot of $\overline{M}$ as a function of

$$\frac{\pi^2 D_c t}{r_o^2}$$

A number of solutions are given by Newman (6). This method of evaluation is outlined in the following section.

Materials and Methods

Since it was desired to study only those variables affecting moisture migration within wheat kernels, precautions were taken to minimize

variations in drying conditions. Wheat was dried in a specially constructed cell in which the sample formed a layer three to four kernels deep when at rest. A rapidly moving current of dry, heated air induced a vigorous stirring action in the sample and ensured a high rate of heat transfer. With this arrangement each kernel was fully exposed to dry air at constant temperature. The rate of internal liquid flow, and hence the drying rate, is a function of the temperature of the kernel rather than of the air. As long as evaporation from the kernel is proceeding there must necessarily be a differential between grain and air temperatures which is related to the rate of heat transfer required for evaporation. For this reason diffusion coefficients should be determined at specific grain temperatures.

Wheat Sample. The Thatcher wheat selected for study was of No. 2, second generation, registered seed grade grown in 1952. This ensured that the sample was sound, clean, and pure as to type and variety. Since the wheat showed a protein content of 13.4% and on experimental baking gave a loaf volume of 860 cc. (bromate formula), it was considered as typical of Western Canadian Hard Red Spring Wheat.

Tempering. The wheat was purchased in the summer of 1953 and at the time of tempering the first series of samples for this study it had a moisture content of 10.3%, dry basis. Six months later when a second series of samples was prepared, the moisture content had fallen to 9.6%. To secure the desired moisture levels the required amount of water was added to the sample in a glass container which was then tightly stoppered. After conditioning at room temperature for 3 days the containers were stored in a controlled room at 10°C. to retard mold growth.

Drying. A drying cup made of brass tubing with screened bottom and lid (Fig. 1) was mounted on the outlet pipe from a steam jacketed heat exchanger. Air flows were adjusted to give a velocity of 20 feet per second which produced a vigorous fluidizing action in the sample. Air temperatures were varied by blending heated air from the heat exchanger with unheated air from the main. Suitable pressure reducing valves and flow meters were included to maintain uniform air velocity and temperature.

Tempered samples were warmed from 10°C. by allowing them to stand a day at room temperature. An aliquot of 12–13 g. was taken for determination of initial moisture content and a similar aliquot was used in the drying test. After air temperature and flow rates were set, the sample was placed in position and dried for 19.7 minutes. It

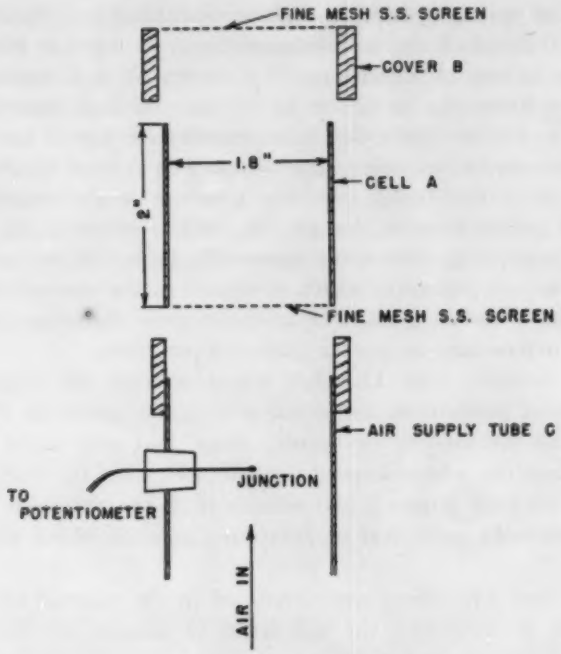


Fig. 1. Drying cell and fittings.

was then promptly transferred to a stoppered bottle and cooled prior to determination of final moisture content.

Moisture. Samples for moisture determinations were first ground in a Hobart coffee mill, moistures were then determined by the 130°C., 1-hour oven method (1). At moisture levels of 15% and higher maximum deviation in replicates was in the order of ±0.1%; at 12% and lower the deviation was ±0.03%. All moisture contents were calculated on a dry matter basis.

Kernel Temperatures. To determine kernel temperatures two methods of measurement were employed. In the first (the cell was first removed from the air stream) a thin sensitive thermocouple was inserted among the grains in the cell. As a check on this procedure the thermocouple was embedded in a wheat kernel which was then dried in the usual manner. Conduction of heat along the thermocouple wires was minimized by threading several kernels on the lead before inserting the junction into the kernel where temperatures were to be recorded. The two methods gave very good agreement. This is shown in Fig. 2 where values obtained using both methods are plotted at intervals during a 20-minute drying period for an air temperature

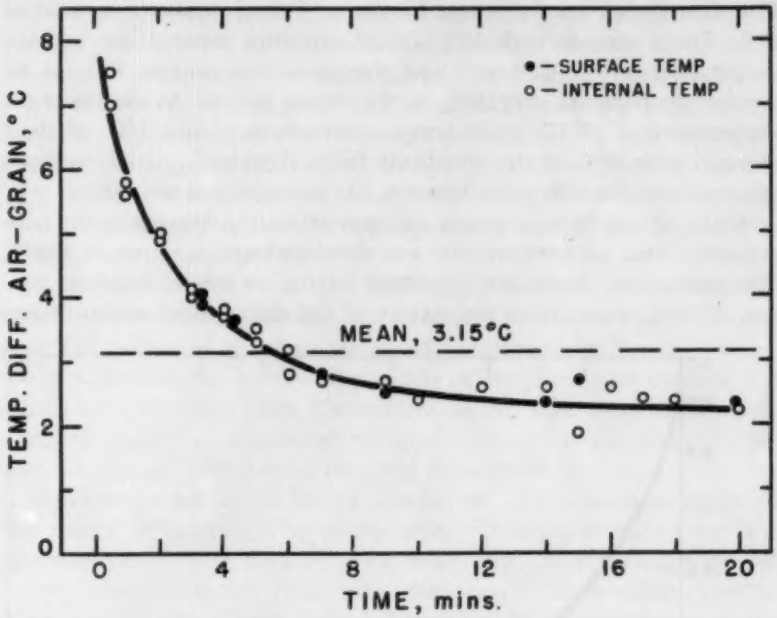


Fig. 2. Temperature difference between air and drying wheat kernel as a function of drying time. Air temperature, 70.5°C. Initial moisture content, 28%.

of 70.5°C. Even at this air temperature the grain was within 1°C. of its average temperature for over 85% of the drying time interval.

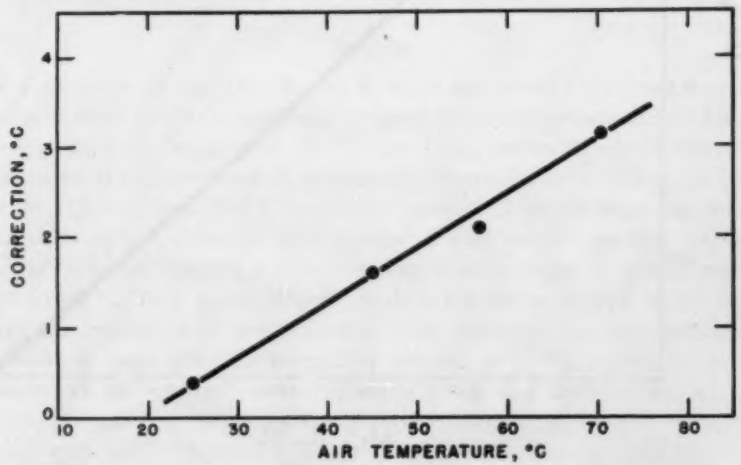


Fig. 3. Mean correction in temperature difference to be subtracted from air temperature to give average grain temperature during drying.

The data shown are for wheat having an initial moisture content of 28%. For a sample with 16% initial moisture content the average temperature was 1°C. lower and the grain was within 1°C. of its average temperature over 95% of the drying period. At the lower air temperature of 45°C., grain temperatures were within 1°C. of their averages over 95% of the 20-minute interval at both initial moisture contents and the difference between the averages was only 0.6°C.

Using this technic, a graph for approximating average grain temperatures from air temperatures was developed and is shown in Fig. 3. The corrections shown are for wheat having an initial moisture content of 28%, since, from the nature of the drying-time moisture-con-

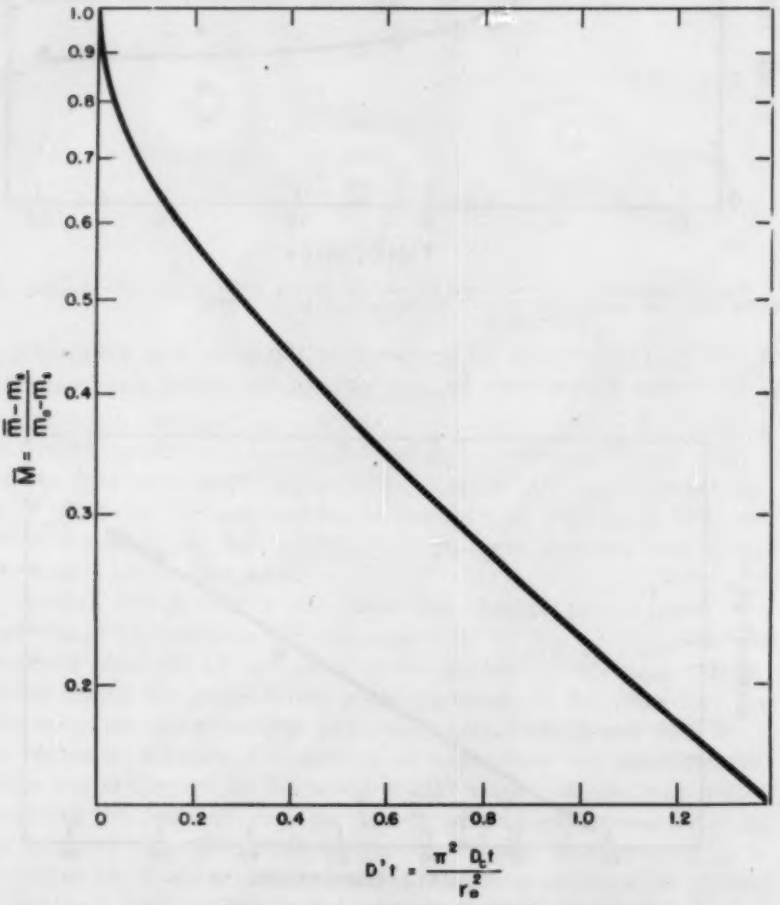


Fig. 4. Plot of $\bar{M}$ vs. D't for evaluation of the diffusion coefficient.

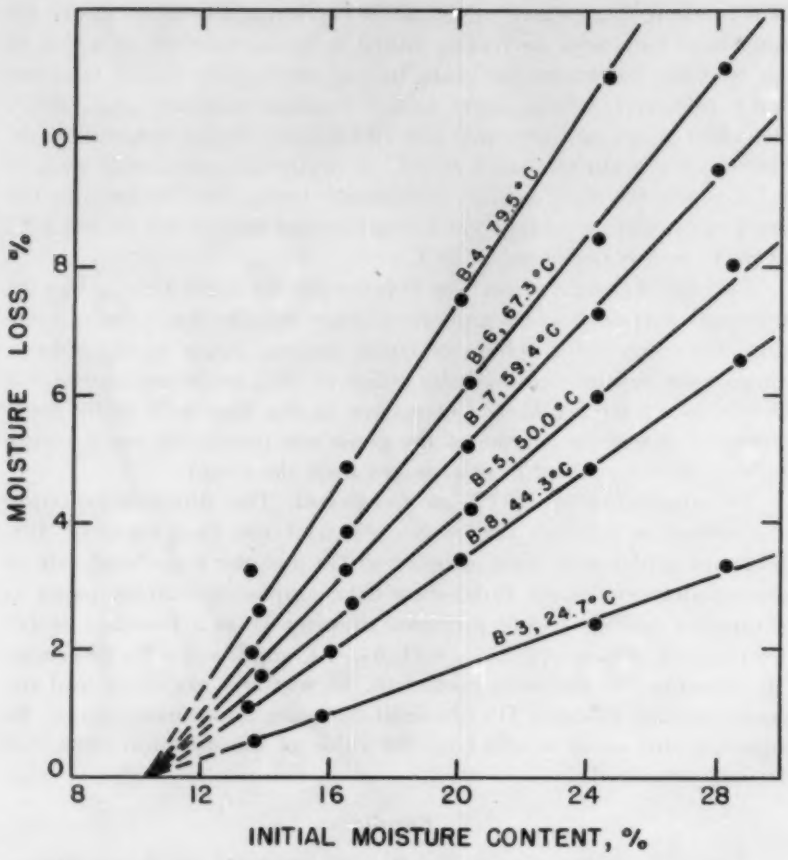
tent relation, it appeared advisable in correcting the temperature for a series of runs with decreasing initial moisture contents to apply to all runs the correction for grain having the highest initial moisture level; moreover, at the lower initial moisture contents (e.g., 16%), the effect upon moisture loss per 19.7-minute drying interval of increasing the grain temperature 1°C. is negligible, amounting to only 0.1% under the most extreme conditions. Using these corrections the error in estimating average grain temperatures should not exceed 1°C. at 80°C. and is negligible at 25°C.

Effective Kernel Radius. The volume per thousand kernels was determined pycnometrically and the average volume was used to compute the radius of a sphere of equal volume. Since kernel volume varies with moisture content the radius at 10% moisture content was chosen as a reference basis. Corrections to this base were easily made since the change in volume of the grain was practically equal to the volume of water added to or removed from the sample.

Calculation of the Diffusion Coefficient. The diffusion equation was solved as follows. The group $\pi^2 D_c t/r_o^2$ was designated by D′t; arbitrary values were then assigned to D′t and the right-hand side of the equation evaluated. From these data, graphs on semi-log paper as illustrated in Fig. 4 were prepared showing $\overline{M}$ as a function of D′t for ranges of values of D′t 0 — 0.14, 0 — 1.4, and 0 — 14. To determine an experimental diffusion coefficient, $\overline{M}$ was first evaluated and the corresponding value of D′t obtained from the appropriate graph. By equating this value to $\pi^2 D_c t/r_o^2$ the value of the diffusion coefficient D_c was computed.

Results

Two series of samples, A and B, were tempered for drying experiments. Initial moisture contents in series A were approximately 14.8%, 18.6%, 22.4%, 25.7%, and 32.0%, dry basis. Series B, which was run 6 months later, consisted of samples at approximately 13.8%, 16.5%, 20.4%, 24.3%, and 28.1% moisture content. A graph showing moisture loss in per cent per 19.7-minute drying period plotted against initial moisture content is shown in Fig. 5 for a range of temperatures 25° to 80°C. This figure illustrates data for Series B and shows that with the exception of two points at 14% moisture the data are fitted by straight lines which intercept the abscissa at 10.3% initial moisture content. This compares with values of 9.1% and 10.3% obtained by Jones (4) using a vacuum drying technic and temperatures between 23.5° and 46°C. It would appear that the relation plotted is truly linear down to at least 14% initial moisture content.



$$\overline{M} = \frac{\overline{m} - m_e}{m_o - m_e}$$

Fig. 5. Moisture loss per 19.7-minute drying time at different corrected grain temperatures, as a function of initial moisture content. Wheat of lot B.

Calculated diffusion coefficients for series A and B, together with related data, are presented in Table I. The last four coefficients were computed from data given by Jones (4). Data on weight per 100 kernels facilitated the calculation of kernel radius in this case. It will be noted that, with the exception of the values marked, the surface moisture for all samples in series A and B is 10.3%. In series A the two divergent values were obtained on samples which had been stored for 6 months and had developed mold growth. In series B the divergent values were obtained with runs made on the third and fourth

TABLE I

DIFFUSION COEFFICIENTS AS COMPUTED FROM EXPERIMENTAL DATA

Series and Number	Days after Tempering	Grain Temperature	Surface Moisture	$\overline{M}$	Diffusion coefficient $\times 10^{-6}$ cm ^{2}sec^{-1}
		°C.	%		
Drying period for series A and B, 19.7 minutes					
A-3	30	20.8	10.3	0.840	0.069
A-1	15	27.0	10.3	0.796	0.116
A-2	16	42.5	10.3	0.620	0.440
A-6	184	48.1	12.7[a]	0.532	0.710
A-4	31	67.8	10.3	0.282	2.210
A-5	183	77.6	12.9[a]	0.045	7.200
B-3	7	24.7	10.3	0.823	0.097
B-8	31	44.3	10.3	0.648	0.375
B-2	4	45.5	12.0[b]	0.588	0.536
B-5	9	50.0	10.3	0.576	0.565
B-9	32	52.8	10.3	0.558	0.635
B-7	30	59.4	10.3	0.475	0.952
B-1	3	61.0	12.3[b]	0.363	1.580
B-6	10	67.3	10.3	0.380	1.500
B-4	8	79.5	10.3	0.230	2.770
Data on vacuum drying (Jones, 4) Drying period, 20 minutes					
		23.5	9.1	0.819	0.086
		30.0	9.1	0.749	0.169
		40.0	9.1	0.644	0.359
		46.0	9.1	0.540	0.653

[a] Samples molded.
[b] Measured too soon after tempering.

days after the samples were tempered. Abnormality apparently was caused by nonequilibrium initial moisture distributions. It should be noted that a linear relation between moisture loss per 19.7-minute interval and initial moisture content was obtained for both types of abnormal behavior.

While Jones (4) gives a value of 10.3% moisture content as the intercept with the abscissa for his data at 30°C., the data are just as well represented by using the value of 9.1% and this value was used for calculation of the coefficient in Table I. It should be noted that Jones used three different varieties of wheat. His results therefore suggest that variety has little influence on the value of the diffusion coefficient or on the surface moisture.

The effect of changes in temperature on the diffusion coefficient is shown in Fig. 6 by the linear relationship between the logarithm of the diffusion coefficient and the reciprocal of the absolute temperature. This behavior is typical of an Arrhenius type temperature dependency and may be expressed by an equation of the type

$$D_e = D_o \, e^{-E/RT}$$

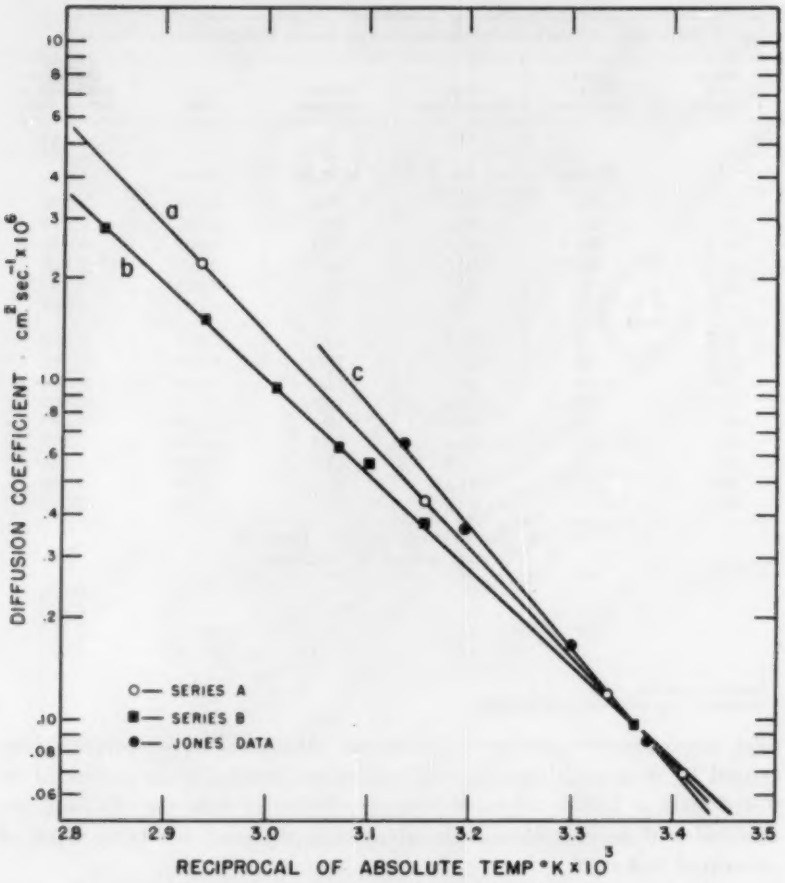


Fig. 6. The diffusion coefficient vs. reciprocal of the absolute temperature.
(a) $D_o = 4.54 \times 10^6 cm^2 sec^{-1}$; $E = 14,600$ cal/mol^{-1}.
(b) $D_o = 2.65 \times 10^6 cm^2 sec^{-1}$; $E = 12,920$ cal/mol^{-1}.
(c) $D_o = 7.85 \times 10^4 cm^2 sec^{-1}$; $E = 16,300$ cal/mol^{-1}.

For series A the energy of activation was 14,600 cal/mol; for series B, 12,920 cal/mol; and for Jones' data the value was 16,300 cal/mol. Since the only difference between series A and B was a time lapse of 6 months, it would appear that the decrease in energy of activation was due to chemical or physical changes taking place in storage. The present study is not sufficiently intensive to permit definitely ascribing a cause. It may be noted, however, that the moisture content prior to tempering for series B was below 10.3%; this may have caused changes due to structural irreversibilities.

As a check on the limits of validity of the experimental diffusion

coefficients the moisture content-drying time relation was studied for a sample of lot B. The initial moisture content was 20.5%. The experiment was carried out at a corrected average grain temperature of 67°C., corresponding to a diffusion coefficient of $1.5 \times 10^{-6} cm^2 sec^{-1}$. Figure 7 shows the predicted curve for a value of the surface moisture

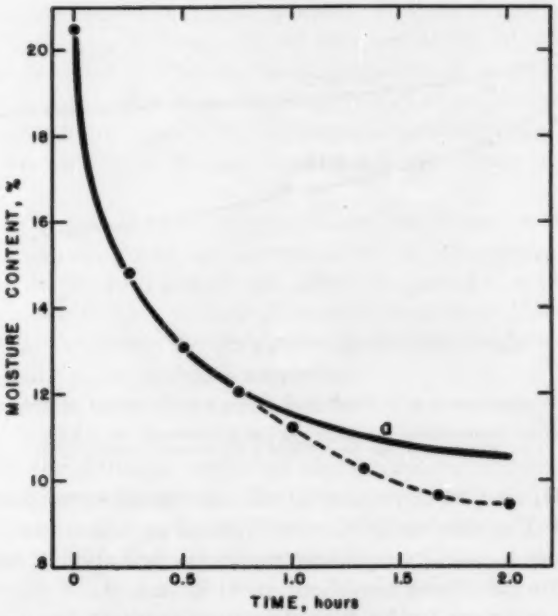


Fig. 7. Moisture content as a function of drying time at 67°C. corrected grain temperature, showing the deviation from the theoretical relation. Initial moisture content, 20.5%. (a) Drying curve for $D_e = 1.50 \times 10^{-6} cm^2 sec^{-1}$, $m_s = 10.3\%$

of 10.3% and also the experimental points. For moisture contents from 20.5% down to 12% the calculated and experimental values agree within the accuracy of the moisture determinations. The diffusion equation predicts that the moisture content should with increasing drying time approach 10.3% as a limiting value. Hence no moisture contents below this value are predicted and the deviation from the predicted curve at some value above 10.3% is bound to occur, since drying does not stop at 10.3% but rather proceeds at a greatly reduced rate.

The new method of estimating diffusion coefficients has not yet been applied to moisture contents below 10.3%. A number of drying time-moisture content curves were determined, however, in order to obtain an indication of the magnitude of the coefficient at these

moisture levels. The wheat used was an untempered lot B sample of 9.65% initial moisture content. Determinations were carried out at temperatures of 81° and 50°C. These are represented by the points plotted in Fig. 8. Drying curves a and b in the figure are the calculated

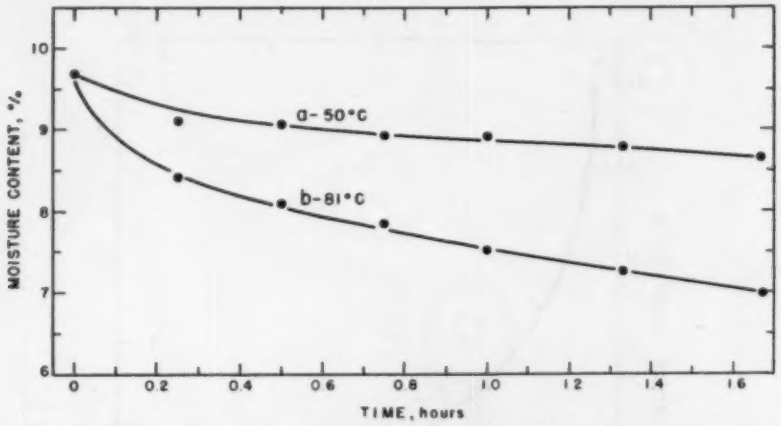


Fig. 8. Moisture content as a function of drying time for wheat of low initial moisture content (9.67%).
(a) Drying curve for $D_c = 0.023 \times 10^{-6} cm^2 sec^{-1}$; $m_s = 5.2\%$.
(b) Drying curve for $D_c = 0.254 \times 10^{-6} cm^2 sec^{-1}$; $m_s = 5.2\%$.

curves at 81° and 50°C. respectively if a surface moisture level of 5.2% is assumed. The data for 50°C. were difficult to fit accurately, since assumed values for surface moisture level between 4 and 6% fitted equally well. The calculated coefficient at 81°C. was $0.254 \times 10^{-6} cm^2 sec^{-1}$, while the coefficient for lot B in the range above 10.3% moisture was $3.1 \times 10^{-6} cm^2 sec^{-1}$. At 50°C. the corresponding coefficients were $0.023 \times 10^{-6} cm^2 sec^{-1}$ and $0.565 \times 10^{-6} cm^2 sec^{-1}$.

The diffusion coefficients calculated from the moisture content-drying time data are subject to some uncertainty and may be appreciably in error. They are included here only to indicate and emphasize the magnitude of the difference in drying behavior above and below the 10% moisture level. In passing through this transition zone from lower to higher moisture levels, diffusion coefficients appear to increase by a factor of 10 to 25 times in the temperature range 50°–80°C.

Discussion

It has been generally recognized that some portions of moisture in damp wheat are held more firmly than others. As a result numerous references to "free" and "bound" water appear in the literature, but the limits appear to be determined by the nature of the experiment.

Differential heat curves calculated by one of us (H.A.B.) from desorption isotherms indicate that the net differential heat of desorption decreases continuously from zero moisture content and approaches zero at a moisture content of 32% without any sharp discontinuities in the relationship (results to be published). Under these circumstances it would appear that the very large and sharp decrease in value of the diffusion coefficient as the moisture content drops below 10% must be largely due to a change in the mechanism of diffusion. It is not to be expected that the diffusion coefficient at moisture contents below 10% is constant over any extensive range of moisture levels, for the coefficient should approach zero at zero moisture content. There does, however, appear to be some moisture independency in the range 7–10%.

The only apparent error of appreciable magnitude in the evaluation of the diffusion coefficient is that due to the assumption of spherical particle shape. This should not affect the practical application of the results, since wheat grains of all varieties have practically the same particle shape. A method for evaluation of the diffusion average-shape-factor constant could probably be devised.

In the present work no attempt has been made to study the effects of relative humidity, air velocity, or reduction of atmospheric pressure. While the diffusion coefficient should not be influenced by external conditions, changes in the drying rate may be effected by changes in the surface moisture. This is indicated by the lower surface moisture level in Jones' vacuum experiments. It has been shown by previous workers (8) that air velocity has little effect on the drying rate, unless, presumably, greatly reduced. The influence of relative humidity was also found to be small. It may be expected that relative humidity should have little influence unless it exceeds the equilibrium value corresponding to the surface moisture content.

The present work demonstrates that in the range of practical drying, moisture migration at a given temperature is accurately portrayed by the diffusion equation with constant values of the diffusion coefficient and surface moisture content. A knowledge of the diffusion coefficient and its temperature dependency permits the prediction of grain temperature and drying time. For general application a statistical mean value of the diffusion coefficient-temperature relationship should be determined for wheats of different variety and history.

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STUDIES ON TECHNICS FOR RECONSTITUTING FLOURS[1]

C. C. WALDEN[2] AND W. B. MCCONNELL[3]

ABSTRACT

Flour was separated into gluten, starch, and a third fraction combining the tailings starch and water solubles. The starch was air dried and the other two fractions lyophilized. Experiments were done with gluten separation times of 75 minutes and 30 minutes at room temperature and 38 minutes at 0°C. Flours reconstituted from the dry fractions gave optimum loaf volumes about 15% lower than the original flours. Damage to baking strength was reduced to within the experimental error of the baking test when the sodium chloride normally included in the baking procedure was incorporated in the mixing water. Substitution experiments suggested that, in the absence of sodium chloride, there were changes in more than one flour fractions.

The method may be of value for studying the role of flour components in baking and the effect of different methods of separation and drying on flour fractions.

Separation of flours or doughs into fractions by washing has been used extensively in studies on the role of gluten (2, 3, 11), starch (15, 16), tailings starch (15), and water-soluble materials (7, 12, 13) in the baking process. Washing procedures were also used in recent studies concerning the role of flour fractions in bread staling (5, 14). These investigators attempted, in so far as was possible, to prevent damage to the baking strength of the flour fractions. In most studies the separations were made with water, but Pence, Elder, and Mecham (12, 13) used 0.1% neutral phosphate buffer as recommended by Dill and Alsberg (6) for quantitative gluten separations.

Various procedures have been used to safeguard the individual fractions during the fractionation and reconstitution. Gluten has been reincorporated into dough as wet gluten (12, 15), or after being frozen in chunks or strips (5, 13). It has been recombined with flours after air drying at room temperature (2, 3, 7), after spray drying (10), and after lyophilization (1, 9, 14). Tailings starch has been dried in air at room temperature after precipitation with organic solvents (5) and by lyophilization (14). The tailings starch is frequently not isolated as a fraction distinct from starch because of its tendency to case harden during normal drying. Starch gives little difficulty in drying and is usually air dried at room temperature and rubbed

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through a fine screen while still slightly damp. Processing the water-soluble fraction requires a method of reducing the large volumes of water. This may be accomplished by evaporation at room temperature and reduced pressure (7, 12), by lyophilization (14), or by a combination of both methods (4, 13).

Reports of the damage occurring with various separation and reconstitution technics are meager. Early investigators (7, 15) found no damage, but Harris (8) indicated that some alteration occurred in gluten air-dried at room temperature (2, 3). Adams (1) reported that larger volume responses were obtained by supplementing a low protein flour with lyophilized gluten than with air-dried or vacuum oven-dried gluten. That flours are changed after separation and recombination is also shown by the work of Bechtel and Meisner (5). A special sponge-type formula was required to make their reconstituted flours yield baking data equivalent to the original flour. In the only complete separation by freeze-drying for which data are available, loaf volumes from reconstituted flours are approximately 15% less than those from the original flour.[4] Because of the possible damage to components during flour fractionation and recombination, the reconstitution technic has necessarily been used on a comparative basis only.

This study was undertaken to devise a basic procedure for fractionation and recombination of flour with minimum damage to baking strength. Such a procedure would open the way for many investigations about the role of individual flour fractions in the baking process and of the effects of various treatments on their baking quality.

Materials and Methods

Flours. A number of unsupplemented, commercial straight flours, milled from Western Canadian hard red spring wheats, were used. These had protein contents as indicated in Tables I and II, and ash contents ranging from 0.40% to 0.45%.

Separation Procedures. Two methods of separation were used, the one comprising a batter process (9), the other a dough kneading procedure. In the batter process 1300 g. of flour were mixed for 15 minutes in a Hobart mixer with 1040 ml. of water. The resulting batter was slurried by vigorous mixing in an additional 7600 ml. of water. The slurry was passed through a 50-mesh screen, and the gluten which collected thereon was washed with four 500-ml. aliquots of water. The time elapsed between wetting of the flour and recovery of the gluten for drying was 75 minutes. In the dough kneading pro-

[4] N. Prentice, L. S. Cuendet, and W. F. Geddes: private communication, 1954.

cedure, 1300 g. of flour were mixed with 780 ml. water for the minimum time necessary to form a smooth dough. The starch and solubles were kneaded by hand, from the dough ball, in 8 liters of water and the remaining gluten subsequently washed in four 1-liter portions of water. This process involved either 30 minutes at room temperature or 38 minutes at 0°C. between flour wetting and recovery of gluten for drying. All separations with sodium chloride present were made with the dough kneading technic.

Sodium chloride (35.75 g. or 1.75%, flour basis) was normally dissolved in the 780 ml. water used to form the dough. In one instance, the sodium chloride was dry mixed with the flour and in another, dissolved in the 8 liters of wash water.

In all experiments the starch and tailings were separated from the water solubles in a 12-inch solid bowl Tolhurst centrifuge ($1000 \times G$). When the starch suspension was added at the rate of about 400 ml. per minute the starch conveniently collected in the lower of the three rings in the bowl, and the tailings collected in the middle ring.

Drying Methods. The gluten was spread as uniformly as possible in trays of a Proctor and Schwartz freeze-drying unit, covered with water and immediately frozen. Drying was controlled so that the ice mass remained below —15°C. and the dried product did not rise above 30°C. The starch was air dried and while still damp was rubbed through a 20-mesh screen. To reduce handling the tailings were suspended in the water-soluble fraction and the combined materials were frozen and lyophilized.

The dried gluten and the tailings-solubles were individually ground in a Wiley mill to pass a 1-mm. sieve.

Reconstitution. Flour fractions were recombined with a minimum change in the ratio in which they were derived from the original flour and to give a composition as near as possible to that of the original flour. One series of reconstituted flours was prepared so that each flour contained only one fraction from a separation where sodium chloride was not used, that fraction being different for each flour. A preliminary mixing was done in a small McLellan mixer and the flours were then ground on the smooth rolls of an Allis-Chalmers experimental mill to pass a No. 50 screen.

The protein contents of the reconstituted flours, calculated from the nitrogen contents of the individual fractions and their reconstitution ratio, were compared with the results obtained by direct analysis.

Sodium Chloride. When sodium chloride was used in the flour

separation, the sodium chloride content of the flour and flour fractions was determined by analyzing for soluble chloride (4).

Baking Formulas. All baking data were secured with the following formula: 100 g. flour (14% moisture basis), 3 g. yeast, 5 g. sucrose, 1.75 g. sodium chloride, 3 g. shortening, 4 g. nonfat dry milk solids, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt, sodium sulfite, and/or potassium bromate as indicated and absorption to suit.

When sodium chloride was used in the initial separation, it was normally omitted from the baking formula. In the series in which each flour contained one fraction from a separation made with no sodium chloride, any sodium chloride deficiencies indicated by analytical data were corrected by appropriate additions at the time of dough mixing.

The original flour, in each case, was baked with increments of potassium bromate to the formula. Except in one instance when potassium bromate only was used, each reconstituted flour was baked in two series, one with increments of potassium bromate, the other with increments of sodium sulfite. For each flour, the loaf with the maximum volume was selected as the optimum loaf, since other characteristics of this loaf were equivalent or superior to those of other loaves produced from the same flour. Loaf volumes tabulated in this paper are averages of at least two duplicate loaves which agreed to within 15 cc.; values are regarded as correct to at least 5%.

Percent flour "damage" has been arbitrarily defined as the difference between the maximum loaf volumes from the original and reconstituted flours, expressed as percent of the loaf volume from the original flour.

Results and Discussion

Fractions and Reconstituted Flours. In Table I are shown moisture and protein contents and reconstitution data for a typical separation. Material losses of about 2% were always encountered. Comparison of experimental and calculated protein values of the reconstituted flours with the protein content of the original flour was an additional check on the recombination.

Types of Separations. Examination of separation conditions was based on the possibility of the damage being enzymatic in nature and involving the gluten fraction. Two initial separations were made with the batter process, only one of the reconstituted flours being baked with increments of both potassium bromate and sodium sulfite. Of the six subsequent separations with the dough kneading pro-

TABLE I

TYPICAL SEPARATION AND RECONSTITUTION DATA FOR A STRAIGHT GRADE FLOUR

Material	Yield[a]	Moisture[b]	Protein[c]	Reconstituted Flour[d]
	%	%	%	
Original flour	100.0	13.0	14.8	...
Gluten fraction	21.5	5.0	59.8	23.2
Starch fraction	39.1	10.7	0.48	44.7
Tailings — solubles fraction	37.1	6.2	4.8	40.6
Reconstituted flour:				
Experimental	97.9	7.6	14.5	
Calculated		7.8	14.9	

[a] Moisture-free basis.
[b] Actual moisture content of experimental materials.
[c] 14% moisture basis.
[d] The ratio of recombination of experimental fractions is calculated on the basis of the moisture contents shown in column 3.

cedure, one was at room temperature, a second was the only separation made at 0°C., and the other four were made with sodium chloride present, dissolved in the dough water and in the wash water, and dry mixed with the flour. The details of the various separations, together with analytical and baking data for the original and reconstituted flours, are given in Table II. The first separation with the batter

TABLE II

PROTEIN CONTENTS, BAKING DATA, AND DAMAGE FACTORS FOR ORIGINAL AND RECONSTITUTED FLOURS CORRESPONDING TO VARIOUS METHODS OF SEPARATION

Separation No., Type and Conditions of Separation	Protein Content (14% m.b.)		Improver[a] Addition		Optimum Loaf Volume[b]		Damage[c]
	Original	Reconst.	Original	Reconst.	Original	Reconst.	
	%	%	*mg/100 g flour*		*cc.*	*cc.*	%
1. Batter	12.2	12.6	0.5B	0.0	830	700	15.6
2. Batter	12.5	12.2	0.0	2.0S	790	680	14.2
3. Kneading	12.5	12.2	0.3B	5.0S	815	690	14.3
4. Kneading at 0°C.	14.8	14.8	1.0B	4.0S	890	750	15.0
5. Kneading, NaCl	14.1	14.3	2.0B	1.0B	850	830	2.5
6. Kneading, NaCl	14.3	14.3	1.0B	0.5B	870	820	5.7
7. Kneading, NaCl in wash water	14.4	14.4	1.0B	0.0	830	755	9.2
8. Kneading, NaCl dry-mixed with flour	14.1	14.1	1.0B	2.0S	920	855	7.2

[a] B = potassium bromate; S = sodium sulfite.
[b] Maximum loaf volume obtained from original and reconstituted flours by baking each flour with increments of sodium sulfite and/or potassium bromate.
[c] Difference between optimum loaf volumes from original and reconstituted flours, expressed as percent of loaf volume from original flour.

process was repeated, because originally the reconstituted flour was baked only with increments of potassium bromate and all levels of improver resulted in decreased loaf volume. Although reconstituted flour from a second batter separation was baked with increments of both bromate and sulfite, no significant reduction in baking damage resulted. Reduction of separation time by use of the dough kneading procedure, either at room temperature or in a cold room (separations 3 and 4) failed to reduce damage. With sodium chloride present, dissolved in the water used for doughing up the flour, the 2.5% damage obtained in separation No. 5 is within the limits of experimental error. Similar data were obtained in separation No. 6 and are also shown in line 2, Table IV. In the latter case the damage was calculated as 0.9%. In separations 7 and 8 where sodium chloride was dissolved in the wash waters and dry mixed with the flour respectively, the higher percent damage suggests that less damage occurs when there is a more immediate contact of dissolved sodium chloride with flour solids. It was shown that the damage recorded in the last column of Table II cannot be attributed to lyophilization. A stiff flour- water dough, mixed a minimum time, was frozen immediately after mixing and lyophilized. The lyophilized flour, after regrinding, gave an optimum loaf volume of 855 cc. compared to 850 cc. for the original flour. Improver responses, and the loaf characteristics, crumb color, crust color, shape, and texture were very similar to those of the original flour.

In general, all those optimum loaves, whose volumes are recorded in Table II, did not differ appreciably in crust color and crumb color, and although the textures from satisfactory reconstitutions and original flours were equally good, damage, as measured by decreased loaf volume, was always accompanied by a marked loss in texture. External and internal appearances of loaves from the original flours and flours reconstituted after separation with and without sodium chloride are shown in Fig. 1.

Action of Sodium Chloride. The distribution of sodium chloride during a fractionation, made with the dough kneading technic, together with the corresponding yield data, are given in Table III. While most of the sodium chloride is in the solubles fraction, an appreciable portion is in the gluten fraction. This may be partly due to occlusion, but since the total volume of the tailings-solubles fraction is about 10 times that of the wet gluten, and since the crude gluten was washed four times with sodium chloride-free water, it appears that chloride is preferentially retained by the gluten.

The compositions of the reconstituted flours containing single

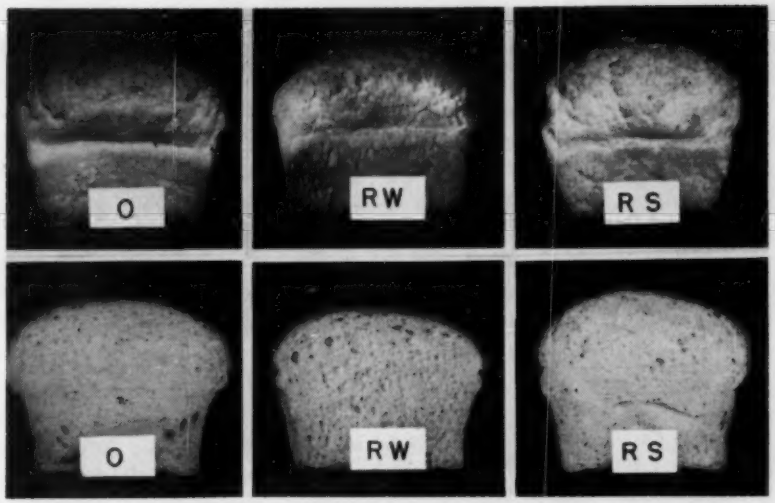

Fig. 1. External and internal characteristics of experimental loaves. O, original flour; RW, reconstituted from separation with water; RS, reconstituted from separation with sodium chloride solution.

replacements with fractions from a separation in which sodium chloride was omitted are given in Table IV. All separations were made with the dough kneading technic. The sodium chloride contents and the baking data for the original and reconstituted flours are also included.

The amount of damage shown individually by these flours containing a substituted fraction is of the order of possible experimental baking error. The limited accuracy of the methods therefore makes it impossible to conclude that changes contributing to damage have

TABLE III

DISTRIBUTION OF SODIUM CHLORIDE IN DRY FLOUR FRACTIONS

Material or Fraction	Sodium Chloride Content
	%
Flour as received	0.20
NaCl added to mixing water (as percent of flour weight)	1.75
Gluten (19.2% of flour weight)	1.02
Starch (36.5% of flour weight)	0.35
Tailings-solubles (42.2% of flour weight)	3.43
Reconstituted flour (97.9% of flour weight)	1.81[a]

[a] Calculated from yield and sodium chloride content of flour fractions.

TABLE IV

COMPOSITION AND BAKING DATA OF FLOURS RECONSTITUTED AFTER SEPARATION WITH SODIUM CHLORIDE, WITH INDIVIDUAL SUBSTITUTION OF FRACTIONS OBTAINED FROM SEPARATION WITHOUT SODIUM CHLORIDE

Fraction Substituted	Gluten	Starch	Tailings-Solubles	Protein[a]	Sodium Chloride Content[a]	Improver[b] Addition	Optimum Loaf[c] Volume	Damage[d]
	%	%	%	%	%	*mg/100 g flour*	*cc.*	%
Original flour	...	...	...	14.3	...	2.0B	910	...
Reconstituted (no substitution)	20.8	49.2	30.0	14.7	1.73	0.0	900	0.9
Gluten	21.9	48.1	30.0	14.3	1.70	0.0	865	4.9
Starch	20.9	46.7	32.4	14.4	1.84	1.0S	875	3.8
Tailings-solubles	21.6	49.3	29.1	14.3	0.45	1.0S	855	5.5

[a] 14% moisture basis.
[b] B = bromate; S = sulfite.
[c] Maximum loaf volume obtained from original and reconstituted flours by baking each flour with increments of sodium sulfite and/or potassium bromate.
[d] Difference between optimum loaf volumes from original and reconstituted flours, expressed as percent of loaf volume from original flour.

occurred in all three fractions. However, from previous consistent observations that a decrease of about 15% occurs with reconstitutions in which no sodium chloride was used, it would appear that more than one of the fractions is contributing to the damage.

General Discussion

When these experiments were planned, it was not anticipated that reconstituted flour identical with the original flour could be obtained. Numerous changes, particularly those of an enzymatic nature, are initiated when a dough is made. Mixing and separation handling may be expected to alter the state of oxidation of the gluten proteins. To make adequate comparisons, both original and reconstituted flours were baked at various improver levels and the optimum loaves from each flour compared.

These studies, as well as those previously reported, show that flour made into a dough with water cannot be fractionated without damage to baking quality. Contrary reports may be due to inadequate baking formulas or to the dependence of damage on loaf size. Damaged reconstituted flours yield micro loaves comparable to original flours but give smaller volumes when baked as larger loaves.[5]

[5] See footnote 4.

The damage to baking quality apparently occurs so rapidly during flour fractionation that reducing the time and temperature of the process are without measurable effect.

The use of salts, particularly sodium chloride, for the quantitative recovery of gluten is not new. Dill and Alsberg (6) recommended a 0.1% neutral phosphate solution for gluten determinations, although sodium chloride was almost as effective. Since sodium chloride decreases the solubility of flour nitrogen (17), its effect in gluten estimations will be to reduce gluten dispersion. This should not, in itself, be a critical factor where all components of the separation are recovered, but it demonstrates the effect of sodium chloride on the behavior of gluten. Without baking studies, Dill and Alsberg (6) reported differences in the physical properties of gluten, when salts were absent.

Another possible explanation of the role of sodium chloride in preventing damage during flour fractionations may be its action as a proteolytic inhibitor. If this is so, flour proteins from fractions other than the gluten are also being damaged. Although conclusions cannot be drawn on the mechanism of its action, the results show that sodium chloride is a desirable agent for preventing damage during flour fractionation. Since sodium chloride is a normal ingredient of bread doughs, its use does not interfere with subsequent baking tests on the products. Damage is minimized if the sodium chloride is dissolved in the absorption water.

The described technics may be useful in investigating damage to flour fractions arising from different methods of separation and drying, and may help in further elucidation of the role of various flour components in the baking process.

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BOOK REVIEWS

Indian Food Laws. Compiled by N. V. R. I. Yengar, B. K. Sur and G. T. Kale. 220 pp. Central Food Technological Research Institute, Mysore, India. 1954. R. S. 4/8.

In India, 22 of the 27 State Governments have food laws under which standards for several foodstuffs are prescribed. Considerable differences exist in a number of the standards adopted by the various States and the present volume was compiled to serve as a basis for the development of uniform standards.

The book summarizes the history, operation, and enforcement of food laws in the different states in India. It gives definitions and standards for various foodstuffs and lists permissible additives such as colors, flavors, and preservatives. A number of tables are included in which the chemical standards prescribed for different commodities in the various States are compiled for ready comparison.

Information relating to the quality and standards of fruit and vegetable products under the Fruit Products Order of 1947 is not included, as they are to form the subject matter of a separate monograph.

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Advances in Food Research, Volume V. Edited by E. M. Mrak and G. F. Stewart. x + 538 pp. Academic Press, Inc., New York, 1954. Price $11.50.

This volume of *Advances in Food Research* represents the latest efforts on the part of the editors to attain their objective, which they define as "the coordination and integration of food research to promote an orderly and systematic development of scientific knowledge in this important field." Examination of the articles selected for presentation in this volume indicates that this goal has been achieved in a highly commendable fashion.

The review by Watts on oxidative rancidity and discoloration in meat deals with a subject that is of timely importance to the food industry. The author's critical appraisal of the literature does much to clarify the many conflicting observations one finds in the literature pertaining to this problem. Particular emphasis is placed on the fundamental causes of oxidative rancidity and discoloration induced by such chemical factors as heme pigments, unsaturated fatty acids, pH, salts, metals, etc., and such physical agents as oxygen tension, light, and temperature. The section on antioxidants should be of more than passing interest to the practical meat technologist.

The chapters on the sugar-sulfite reaction, by Gehman and Osman, and the preservation of fruits and vegetables by sulfite treatment, by Joslyn and Braverman, relate to the fundamental and practical aspects of using sulfites in foods. In the reviewer's opinion a single chapter on this general subject would have sufficed, since considerable duplication of material was quite apparent throughout both chapters. (By actual count at least 40 references were duplicated.) Both chapters deal with the chemistry of the sulfites and their addition products, analytical procedures, the role of sulfites in the browning reaction, and the specific application of sulfites in the processing of various food commodities.

The inclusion of a chapter on statistical methods in food research, by Ostle and Tischer, is a reflection of the increased emphasis being placed on the use of statistics in the design and interpretation of experimental work. The treatment afforded this subject may appear rather advanced to the uninitiated, and for this reason one can only hope that this chapter will engage the interest of those who have the most need for a knowledge of statistics. The utility of this chapter is considerably enhanced by the liberal use of examples of experiments extracted from the literature and subjected to detailed statistical treatment.

Bate-Smith's chapter on flavonoid compounds is a masterly treatise which should be invaluable to those interested in the distribution and properties of natural food pigments. Also of importance with respect to food pigments is an understanding of their effects on the appearance of foods. This subject is thoroughly covered by a chapter on color problems in foods, by Mackinney and Chichester. One cannot help being impressed by the enormous amount of work that has been done in this field in an attempt to put color measurement on a sound scientific basis.

Amerine has done a monumental piece of work in reviewing the vast amount of literature dealing with the composition of wines. Valuable supplementary information relating to methods of analysis and their interpretation is likewise included in this chapter. Much of this material has been extracted from foreign literature, thus providing information which may not be otherwise accessible to many workers in this field.

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Carotenoids—Their Comparative Biochemistry, by T. W. Goodwin. The Chemical Publishing Co., Inc., Brooklyn 2, N.Y., 1954. Price $7.00.

This book summarizes the biochemical aspect of the occurrence, formation, and function of carotenoids in higher plants, fungi, bacteria, and all animals. It brings together in a well-organized volume a wealth of information concerning the distribution of these pigments in diverse plant and animal materials. Broad conclusions on basic concepts are well documented. The biochemical approach makes the book particularly valuable to the biochemist and physiologist, because known biochemical and physiological findings are pointed out. Also, areas of the field that are poorly known or unexplored are noted. The book will also contribute materially to the zoologist, entomologist, microbiologist, and botanist, because of (1) the wide number of living materials covered and (2) the complete bibliography appended to each chapter.

The book contains four chapters on plants, six on animals, and one on conversion of carotenoids into vitamin A. The coverage of original articles has been made with a thoroughness usually found only in reviews of limited subjects.

The author has done an exceptional job in emphasizing the similarities in the occurrence and behavior of these pigments, thus bringing into an orderly system many isolated and often miscellaneous observations. He points out that these widely occurring substances undoubtedly have more functions than merely providing color and vitamin A precursors for animals. When we find out how these pigments are formed and metabolized we will have made significant advances in our understanding of living processes.

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Color in Foods: A Symposium, ed. by Kenneth T. Farrell, Joseph R. Wagner, Martin S. Peterson, and Gordon Mackinney. vi + 186 pp. Published by The Advisory Board on Quartermaster Research and Development, National Research Council, Washington, D.C., 1954. Copies are available gratis at the Quartermaster Food and Container Institute for the Armed Forces, 1819 West Pershing Road, Chicago 9, Illinois.

This paper-bound volume represents the edited transcript of a symposium on Color in Foods convened in November of 1953 and sponsored jointly by the National Research Council and the Quartermaster Food and Container Institute. The symposium consisted of a series of papers with considerable discussion of the problems raised. Readers with some background in methods of defining color and the instrumentation involved will find this an excellent up-to-date review of current problems in dealing with color in foods. For those who are beginning to work in this area

it is perhaps of rather limited immediate usefulness as a preliminary source book, since most of the contributing authors assume a working knowledge of the various systems of defining color.

Four main sections deal with: color and its relationship to food investigations; color measurement in relation to commodities and consumer interest; instruments for the study of color; and measurement of color and color differences in relation to quality. The individual papers are generally excellent, and the topics are well knitted together by the discussion. The psychology of color preference in foods is discussed, as well as the chemical significance of color in relation to changes taking place in perishable or preserved foods. The compromises which must be made to put color grading of certain commodities on a production-line basis get a thorough airing along with, in some cases, a fairly detailed description of the instruments which have been developed for this purpose. There is an informative discussion of the comparative usefulness of the various instruments available for color measurement, and a number of specific discussions of the use of some of these instruments to evaluate color for a wide variety of commodities.

To all those presently wrestling with this difficult monster of color measurement, this book can be a very useful and sympathetic companion. It records limited successes in some fields, and indicates the current lines along which progress is being made in others. The discussion provides answers to many queries that arise in dealing with color problems which it would be difficult to find elsewhere, and forms an invaluable part of the book. The editors are to be complimented on the arrangement of the material; the sequence of topics dispels, to a large extent, the sense of discontinuity so often unavoidable in books of this sort.

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Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see
Cereal Chem. **30:** 351–352 (1953).

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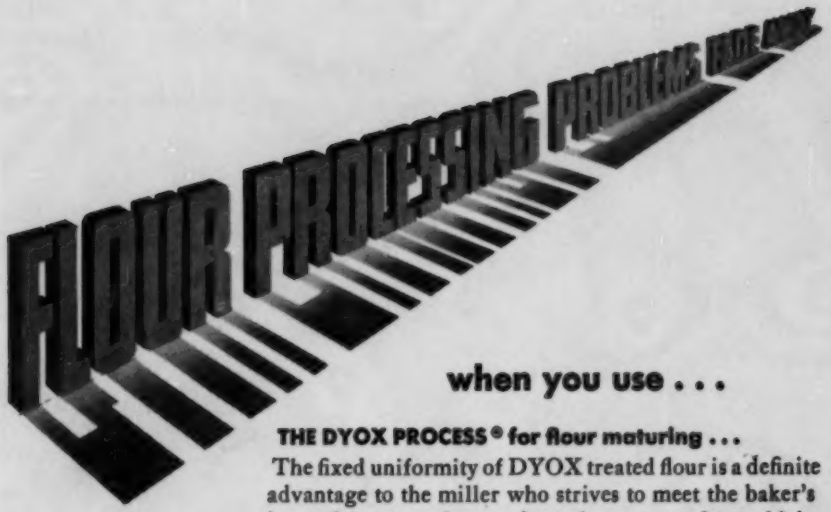

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Presentation

of the

Thomas Burr Osborne Medal

to

R. M. Sandstedt

by the

American Association of Cereal Chemists

May 26, 1954

Supplement to Cereal Chemistry
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RUDOLPH M. SANDSTEDT

REPORT OF THE THOMAS BURR OSBORNE MEDAL AWARD COMMITTEE

GEORGE GARNATZ, *Chairman*

The presentation of the Thomas Burr Osborne Medal this year takes on added significance from the fact that twenty-eight years ago, here in Denver, the idea of a medal sponsored by the American Association of Cereal Chemists was born.

Reference to the history of the Association reveals that at the meeting held in Denver, June 7 to 11, 1926, President Rowland J. Clark proposed that two medals be awarded for each convention; one for the best paper on the industrial application of cereal chemistry, and one for the best paper on fundamental research in cereal chemistry.

The convention voted to give the executive committee power to revise the idea and to take action. As a result, it was proposed that the Association strike a medal to be awarded when it seemed desirable in recognition for noteworthy achievement in cereal research. The medal was designated the Osborne Medal in recognition of the great amount of excellent work which Professor Thomas Burr Osborne had done in cereal chemistry.

A jury of awards was appointed with Dr. C. L. Alsberg as chairman. The design was worked out by Dr. Alsberg and the medal was made under his direction by Shreve and Company of San Francisco. Professor Osborne was designated the first recipient and the Osborne Medal was presented on June 7, 1928, as the Association met in annual convention in Minneapolis.

Since that time, at irregular intervals, and after thorough review of the field for potential candidates, the medal has been awarded to seven other workers so that today the list of recipients reads:

T. B. Osborne	1928
C. H. Bailey	1932
M. J. Blish	1936
C. O. Swanson	1938
R. A. Gortner	1942
J. C. Baker	1945
Betty Sullivan	1948
W. F. Geddes	1950

Since the medal was awarded to Dr. W. F. Geddes four years ago, the Osborne Medal Award Committee has been making a diligent search for eligible candidates. Thorough consideration of the qualifications of an impressive list of candidates led to the selection of Professor Rudolph M. Sandstedt as the ninth recipient of the Osborne Medal. In effect, therefore, Professor Sandstedt becomes the Silver Anniversary Medalist, since the first medal was presented twenty-six years ago.

The Committee, as an instrument of the American Association of Cereal Chemists, is happy thus to give recognition to Professor Sandstedt's long record of high-caliber research in cereal chemistry, which has contributed significantly to the advancement of the science. The addition of his name to the list of distinguished recipients is in keeping with the high standards and fine tradition established for the medal and honors one who, along with his scientific accomplishments, is at the same time a modest, friendly man.

I now call on Dr. M. J. Blish who, in the years past, was a member of that prolific team of Blish and Sandstedt, to recount for us the life and accomplishments of the medalist.

THE CONTRIBUTIONS OF RUDOLPH M. SANDSTEDT TO CEREAL CHEMISTRY

M. J. Blish

What it means to me to serve in this capacity will doubtless be appreciated when I inform you that I am responsible for having started Sandy on his distinguished career as a cereal chemist and that I had the privilege of being associated with him for a period of 17 years. Although, obviously, these facts are merely circumstantial and coincidental, they do add something "extra special" to the personal pleasure that I derive from the well-merited recognition that has been accorded him.

Sandstedt's father and mother were born in Sweden. They emigrated to America in the late 1860's, where they took up one of the earliest homesteads in central Nebraska, at a location 30 miles from the nearest town. Rudolph Marion Sandstedt, born October 17, 1896, has three brothers and five sisters. In due course he attended the University of Nebraska where, in the College of Agriculture, he received the B.S. degree in 1920. Immediately following graduation he accepted appointment as Assistant in Agricultural Chemistry, at a time when the activities of the Department were confined chiefly to analytical service work and to teaching chemistry to undergraduates. During this period Sandstedt started some work on a nutritional research problem involving hand-feeding of hens.

In 1922, it was decided to re-establish an organized program of research with emphasis on cereal technology, and with special attention to the evaluation and utilization of Nebraska wheats, which at that time were being discounted two cents per bushel because of alleged inferiority in baking properties. This decision led not only to my appointment, in 1922, as Chemist of the Agricultural Experiment Station but also, fortunately for me, it provided the opportunity to become associated with R. M. Sandstedt. Sandstedt received the M.S. degree in 1923.

In the early stages of investigations intended to disclose the manner in which baking quality is influenced by factors of heredity and en-

vironment, respectively, the most difficult and perplexing phase of the project was the selection of a laboratory baking test which would adequately serve the purpose. A survey of baking test procedures employed in several mill laboratories disclosed the following facts:

1. No two laboratories used the same formula and procedure.

2. The test loaves reflected a wide range of variation in operative skill and manipulative dexterity.

3. Since the results could be interpreted only in terms of individual preference for specific requirements, the value of the test was limited accordingly.

4. Tests of this type definitely fail to provide an impartial and scientific basis for the analysis and evaluation of a basic material. Specifically, they were, and are, not suitable for an objective characterization of experimentally milled, untreated flours derived from wheats of miscellaneous origin, type, and variety.

Fortunately, the situation was eventually relieved by the adoption, in principle, of the so-called "pup" test, as originally devised by the late Dr. E. E. Werner. The use of this miniature "fixed" straight dough process, in conjunction with appropriate supplementary procedures, proved to be admirably suited to the testing of our experimentally milled flours. It was found to be the only type of test baking procedure in which the elements of manipulative skill and personal preferences were minimized to the extent that it provided a sound basis for impartial and objective characterization. Incidentally, the application of this objective method to Nebraska wheats failed to disclose even the slightest trace of evidence in support of the old notion that such wheats had inferior quality, or that quality was likely to be affected by state boundary lines.

This experience with the laboratory baking test is cited primarily for two reasons. First of all, it proved to our satisfaction that the test, itself, could be made to fulfill the requirements that are normally expected of a scientifically dependable analytical procedure. Secondly, it disclosed specific areas in which research projects might advantageously be formulated and pursued. The latter feature is well exemplified by the series of researches pertaining to the cereal amylases, in which Sandstedt has made so many notable contributions. These investigations originated from some preliminary attempts to subject our experimentally milled flours to the basic pup test, using the quantity of sugar specified in the baking formula employed at that time by Dr. Werner with commercially milled flours. This sugar level proved to be so inadequate that the doughs literally "ran out of gas"

long before the prescribed fermentation time had elapsed, yielding loaves of bread which closely resembled golf balls. Sandstedt promptly demonstrated that these deficiencies in gassing power and fermentation tolerance, which were uniformly characteristic of experimentally milled flours, could be conveniently overcome by the simple expedient of raising the sugar level in the baking formula.

A logical outcome of that experience was the focusing of attention on the importance of diastatic activity and gassing power, especially in test baking by the straight dough method, and thus was initiated the series of cereal amylase investigations to which reference has been made. These investigations, continuing over many years, resulted in the publication of some 25 (or more) papers by Sandstedt and associated workers. They not only yielded new and improved knowledge as to the properties and functions of the cereal and malt amylases, but they also provided analytical and testing methods which have found wide acceptance in all branches of industrial technology involving the enzymic conversion of starches to sugars.

Neither time nor propriety will permit any detailed discussion of the many individual publications that have resulted from these and other researches in which Sandstedt has played a leading part. The titles of his papers will, of course, be listed in the recorded proceedings of this medal presentation ceremony. The topics covered include protein fractionation studies, many aspects of the baking test and their implications, gluten quality, dough-mixing tolerance, maltose fermentation, the effect and significance of oxidizing and reducing agents in baking, environmental and varietal factors as related to baking response, the role of starch in bread staling, reconstituted doughs prepared by separating and later recombining the starches and glutens prepared from different flours, enzymic inhibitors, and detailed photomicrographic studies of wheat starch granules as affected by gelatinization, enzyme digestion, and mechanical treatment. During the course of these investigations, many useful analytical and control methods as well as laboratory appliances and instruments conveniently adaptable for specific purposes have been developed and reported by Sandstedt.

A recent noteworthy accomplishment of Sandstedt and collaborators has been to disprove the theory, which had long been a subject of much controversy, that the effects of oxidizing and reducing agents on dough properties are attributable to inhibition or activation, respectively, of the flour proteases.

Sandstedt was instrumental in developing a useful technique

whereby flour starches and glutens can be separated, then recombined and baked into bread similar to that produced by the original flour. This permits the starches and glutens of different flours to be interchanged, thereby offering a novel approach which has been extensively used in many research laboratories.

In work on the fractionation of gluten proteins Sandstedt introduced several methods having unique and potentially valuable features. It is unfortunate and regrettable that many of these procedures have never been published, for they at least produced factual data which could easily have future value and significance. Incidentally, one type of fractionation method was based upon principles that have been successfully applied, in recent years, to the fractionation of blood proteins. Nevertheless, from this work it was tentatively concluded and reported that there are three main types of gluten protein components, gliadin, glutenin, and an intermediate type for which Dr. Bailey appropriately suggested the name "mesonin." It was also established that a portion of the glutenin fraction occurs as a lecithoprotein or some similar lipid-protein complex, and that the solubility characteristics of glutenin are profoundly altered when the lipid portion of that complex is removed by appropriate means.

Special mention should be made of Sandstedt's participation in a series of investigations which demonstrated the existence of three naturally occurring enzyme inhibitors. One of these, a trypsin inhibitor, exists in raw soybean meal, while flour contains both an amylase and a papain inhibitor. These inhibitors appear to have important industrial and nutritional implications.

A few years ago Sandy began his skillful and painstaking photomicrographic studies of the intimate habits in the private lives of starch granules, with special reference to their behavior during gelatinization, enzyme attack, mechanical disintegration, etc. The remarkable motion picture films resulting from this work have attracted so much nationwide attention and interest, and are now so well known to all cereal chemists and food technologists, that they need no further emphasis in this discussion.

In addition to Sandstedt's achievements in research, he has performed a variety of services from which cereal technology has derived substantial benefits. These include membership on numerous technical committees and active participation in the affairs of our Association, which he has served in the capacities of President, Chairman of the Executive Committee, and Managing Editor of *Cereal Chemistry*, respectively. He has been consultant and advisor for the

National Research Council, for the Quartermaster Food and Container Institute, and for several large industrial concerns.

One of the talents that Sandstedt has frequently displayed is an aptitude for devising simple, practical, and serviceable items of laboratory apparatus and equipment, of which the familiar pressuremeter is a good example. In exercising this talent he has had the benefit of the expert mechanical guidance and know-how of Mr. W. G. Ferris of the National Manufacturing Company. The result of this collaboration and teamwork has been the development and production of a variety of cereal laboratory appliances such as sheeting rolls, molders, mixers, ovens, loaf volumeters, pressuremeters, etc.

In 1928 Sandstedt married Opal Marie Willard, and you can't blame him for that if you know Mrs. Sandstedt. They have three daughters and one son. Among the avocations for which Sandy shows the greatest enthusiasm are gardening, swimming, and Boy Scout work. He has been a Boy Scout swimming instructor for a number of years. Mrs. Sandstedt says that as a gardener he raises enough vegetables and flowers to supply not only the family requirements but also those of their friends and neighbors, and that he can occasionally be seen prowling around the garden after dark, with a flashlight. Some of his tendencies have obviously been inherited by the children. Ruth Ann, a graduate of the University of Nebraska, is a field director for the Camp Fire Girls at Wichita, Kansas. The three older children, along with their father, are Red Cross Water Safety Instructors, while Bob is on the university swimming team. Jean is a university undergraduate, and Karen is a junior high school student. Sandy never wears a hat or topcoat until the outdoor temperature closely approaches zero.

In the early years of my acquaintance with him he enjoyed quite a reputation as an amateur vaudeville entertainer. One of his more spectacular contributions was a fire-breathing stunt involving the use of gasoline. On one occasion he must have either become overconfident or used the wrong kind of gasoline, because he appeared at the laboratory the next morning with his head and face completely covered with bandages which he was not permitted to remove for a week or so. Such an experience immediately caused him to abandon all interest in that particular field of endeavor.

Another sidelight on Sandstedt's many and diversified talents is provided by a quotation from a congratulatory letter which he recently received from a practicing doctor of veterinary medicine: "You perhaps have forgotten that about 28 years ago you saved two students

from flunking organic chemistry. Glen Dunlap and I were in that predicament and one Sunday morning you took us with you to the Ag. campus and drilled us for four hours and after your tutoring we went back and took the exam and came up from a flunk to a grade of 95."

Those of us who have corresponded with Sandy know that his letters are characterized by a strict economy of words. For example, in answer to a letter which I recently wrote him his reply was as follows: "Dear Doc: You are right. Yours truly, Sandstedt." His approach to a research problem usually follows a similar pattern, for he wastes little time and effort in getting down to the main issues involved, by the shortest possible route. Whatever his specific objective may be, he seldom fails to reach it. A man who worked for several years in close association with Sandstedt recently told me that he considers Sandy to be no less than a *genius.* If we accept the definition of genius as "an infinite capacity for taking pains," then surely his exhaustive and persevering photomicrographic investigations on starch, alone, should entitle him to that designation. We may confidently expect many more important contributions from him in the years to come.

PUBLICATIONS OF R. M. SANDSTEDT

SCIENTIFIC PUBLICATIONS

Blish, M. J., and Sandstedt, R. M.
1925 Glutenin—a simple method for its preparation and direct quantitative determination. Cereal Chem. **2:** 57–67.

Blish, M. J., and Sandstedt, R. M.
1925 Viscosity studies with Nebraska wheat flours. Cereal Chem. **2:** 191–201

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1926 An improved method for the preparation of wheat gliadin. Cereal Chem. **3:** 144–149.

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1927 Factors affecting interpretation of experimental baking tests. Cereal Chem. **4:** 291–299.

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1929 Correlation between diastatic power of flour and crust color in the test loaf, and its significance. Cereal Chem. **6:** 121–127.

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1929 Concerning the nature of the protein extracted from wheat flour by hot alcohol. Cereal Chem. **6:** 494–503.

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1932 A note on the use of selenium as a catalyst in Kjeldahl digestion with natural gas heat. Cereal Chem. **9:** 156–157.

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CINEMICROGRAPHIC STUDIES

STARCH GELATINIZATION

1. R. M. Sandstedt and Wendell Hoffman
   The gelatinization of native and modified potato starches.
2. R. M. Sandstedt and Wendell Hoffman
   A comparison of the gelatinizations of some native starches.
3. R. M. Sandstedt and Wendell Hoffman
   Chemical gelatinization of potato starches.

STARCH SWELLING AND SUBSEQUENT DIGESTION

1. R. M. Sandstedt and James Fleming
   The swelling and enzymic digestion of mechanically injured wheat starch granules.
2. R. M. Sandstedt and James Fleming
   Heat gelatinizations with accompanying amylase action.

AMYLASE ACTION ON STARCH GRANULES

1. R. M. Sandstedt and James Fleming
   The structure of wheat, rye and barley starch granules as revealed by enzyme action.
2. R. M. Sandstedt, James Fleming, and J. D. Allred
   A comparison of the action of amylases from various sources on starch granules.

THE MILLING PROPERTIES OF WHEATS

1. R. M. Sandstedt, James Fleming, and J. D. Allred
   A comparison of the milling properties of hard and soft wheats and the effects of tempering. The disintegration of the wheat kernel endosperm when subjected to shearing pressure.

PRESENTATION OF THE MEDAL

R. A. Barackman

Dr. Sandstedt, on behalf of the membership of the American Association of Cereal Chemists, it is my pleasure and honor to present to you the Thomas Burr Osborne Medal in recognition of the outstanding contributions which you have made in your chosen field of structures of cereal grains and cereal starches.

Our congratulations for work well done go with this medal. It is our hope that your efforts in the science and technology of cereal grains will continue to be of large benefit to the Association and to the cereals industry.

ACCEPTANCE OF THE OSBORNE MEDAL

By R. M. Sandstedt

Mr. President, Dr. Blish, Friends:

When I was elected President of the American Association of Cereal Chemists, I thought that I had experienced the greatest possible professional thrill. Now, I know that there is one much greater—to be awarded the Osborne Medal. Tonight I have an extra appreciation of this honor because I can sense your feeling of goodwill and friendliness that goes with it—and I like it.

Through the years I have had the privilege of working with a remarkably fine group of associates. The accomplishments on which this Osborne Medal Award is based are the accomplishments, not of me as an individual, but of this group. Each individual has contributed his ideas and work; together, all of this forms a background of ideas and experience. Through my good fortune this background is mine but the accomplishments coming from it belong to the group. Accordingly, Mr. President, I would like to accept the Osborne Medal more as a representative of a group than as an individual.

PHOTOMICROGRAPHIC STUDIES OF WHEAT STARCH. III. ENZYMATIC DIGESTION AND GRANULE STRUCTURE[1]

R. M. SANDSTEDT[2]

The starch granule has been the subject of a tremendous amount of research. During the period 1820 to 1900 it was a most popular subject for microscopic study. This intense interest is understandable since the starch granule has many properties, peculiar to itself alone, that are still challenging explanation. In 1836 Poggendorf wrote that the starch granule was one of the most studied but least understood of all substances. In 1938 this statement was brought up to date by Badenhuizen (3), who said that after a thorough review of starch granule research it became apparent that we knew little more than was known a hundred years ago. In other words, the starch granule with all of its peculiar characteristics still is largely an intriguing unknown.

Our interest in the starch granule originated from earlier studies of amylase action in doughs (5, 6, 24), which showed that the action in doughs was not comparable to action in boiled starch dispersions. The action in doughs was most readily explained as being dependent on starch susceptibility.

The raw starch granule is exceedingly resistant to the action of digestive enzymes. Figures recently published (28) indicate that the α-amylases may be from 165 to 7000 times as active on boiled starch as on raw starch. A raw starch granule becomes readily susceptible to digestion if it is crushed or mechanically damaged (2, 14, 21). Accordingly, in doughs with a normal enzyme content, the damage that the starch granule received during milling is the main factor in determining amylase action (5, 24).

One obvious approach to the study of the structure of the starch granule and its relation to enzyme action was through direct microscopic observation. Preliminary observations were so particularly informative that for this laboratory photomicrography became a major

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method of research. The development of techniques and equipment for applying photomicrography to the problems of cereal and starch chemistry has made this a most fascinating and profitable field.

Light Diffraction Patterns. As may be imagined from Badenhuizen's statement concerning the lack of progress during the last hundred years, the study of enzyme action on raw starch has involved many difficulties which have caused confusing and contradictory statements in the literature, which in themselves indicate that microscopic observations are subject to misinterpretation. A microscopic observation, or a photomicrograph, of a group of granules does not present a true picture of the granules unless the observer is aware of some of the characteristics of the light-refraction and light-interference patterns that are commonly present. Microscopic observations require interpretation and are subject to misinterpretation.

Ordinarily a photomicrograph is taken with the microscope focused on a feature of particular interest and, since the starch granule has an appreciable thickness, other features may be out of focus; these out-of-focus features need not lead to misinterpretation if the viewer has a knowledge of their characteristics. Figure 1, A, shows the appearance of a field of partially digested granules when the focus (using a 4-mm. objective) was on the holes in the upper surface of the large granule. These holes appear as white dots; however, there also are

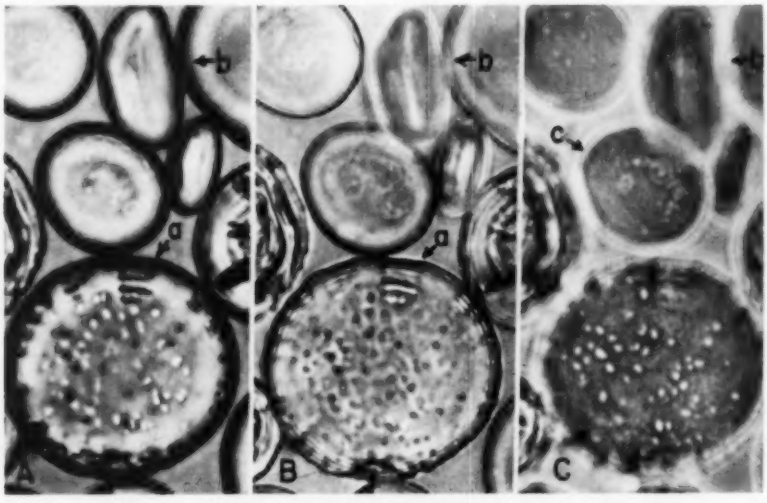

Fig. 1. Effects of variations in focus on the apparent characteristics of starch granules (× 800). Partially digested granules with focus on: A, the upper surface; B, the edges; and C, the lower surface.

poorly defined dark dots which are the out-of-focus holes in the opposite, or lower, surface. The refraction image of features that are below the plane of focus tend to appear black, and the outer edges of the granules appear to be heavy black layers; also, holes and cracks in the interior of the granules are black. Granule *b* (a granule on edge) is in focus; however, the upper end shows a clearly defined double edge—an illusory refraction image known as the Becke line. Figure 1, B, shows the field with the focus on the edge of the large granule, with consequent elimination of the heavy black edges, resulting in a more nearly true picture. The tangential lines *(a)* of enzyme action in the large granule have become white. The holes in the bottom surface are still dark spots, whereas those in the top have become diffused light blotches. Granule *b* is definitely above the plane of focus and consequently shows a multiple Becke line effect. The focus in Fig. 1, C, is on the holes in the lower surface, thus showing the out-of-focus effects in granules that are above the plane of focus. All of the granules have Becke lines which show as bright halos. Granule *b* has an especially clear diffraction ring. The Becke line effect when applied to the image of a point is shown in granule *c* as a point bounded by concentric rings. Features that were seen in the upper planes of focus have disappeared.

Comparison of A, B, and C of Fig. 1 indicates how differences in focusing may cause artifacts. Improper adjustments of the substage diaphragm and condenser also produce similar illusory effects. Such artifacts are difficult to distinguish from reality. Unfortunately they cannot be entirely eliminated when using high magnifications.

However, the major disagreements between authors evidently are due to the investigational methods used. In general, microscopic studies of enzyme action have consisted of observations on "catch samples" taken at intervals from a digest. The interpretation of such evidence is difficult—many structures are produced during digestions that are easily explained if the entire course of their development has been followed but which would be confusing if observed only in a catch sample.

The first microscopical investigations of starch were made by Leeuwenhoek in 1716 (22). He concluded that the granule consists of a kernel or nucleus which is fit for nourishment and an outer, insoluble, non-nutritive envelope. This concept of granule structure still persists, probably because it seems to explain the baffling characteristics of the raw granule. To many observers the light diffraction patterns at the periphery of the granule as shown in Fig. 1 have been

convincing proof of the presence of this external membrane. In addition, the fact that artificial films of various kinds may be superimposed on the starch granule has been misinterpreted to indicate that such a film was an integral constituent of the natural starch granule (10).

Because of the many difficulties involved in keeping a given field of a starch suspension under continuous microscopic observation for long periods of time, few studies have been made showing the continuous action of enzymes. The combination of motion-picture techniques with photomicrography makes possible continuous observations of actions that are much too fast or much too slow for the eye to follow; the swelling of a starch granule that requires only a half-second for completion may be filmed at 64 frames per second, giving 30 individual pictures of the action; or this action may be taken at higher speeds, 1000 frames or more per second, giving a film which, when shown at 24 frames per second, spreads this action over a 20-second period. The digestion of a granule that requires a period of two or three days may be filmed at one frame per minute (or longer intervals if desirable); thus, when the film is shown, the entire condensed action may be seen in two or three minutes. The final action as seen on the screen may vary from one-fortieth as fast as the original to 1500 or more times as fast. In addition the films may be viewed and studied, frame by frame, or may be enlarged and used for illustrating a paper.

Milling Characteristics of Hard and Soft Wheats

Endosperm Cells. As a background for the discussion of the characteristics of starch granules, it is well to recall the structure of the endosperm of the wheat kernel. The endosperm is, for the most part, composed of elongated thin-walled cells that are packed with starch granules.

The photomicrographs in Fig. 2[3] show the variations in segregated endosperm cells as they are found in a hard wheat flour (11, 15, 20) or in a single kernel of wheat. A comparison of these flour particles with pictures of endosperm cells taken at various stages in the development of the wheat kernel (23) leads to the conclusion that the variations represent differences in maturity, even though they all may come from a single kernel. The peripheral layer of endosperm cells (the

[3] The great difference between the refractive index of starch (1.53) and water (1.33), while making possible the visualization of details of a single starch granule, allows too little light transmission for satisfactory observation of flour particles. Detailed photomicrographs (as shown in Fig. 2) may be obtained from suspensions of flour particles in glycerin (refractive index 1.47). The particles on first wetting with glycerin show no detail beyond the particle outlines; however, after a period of soaking, the differences in the refractive indexes of protein and starch become apparent, allowing the starch granules to be seen.

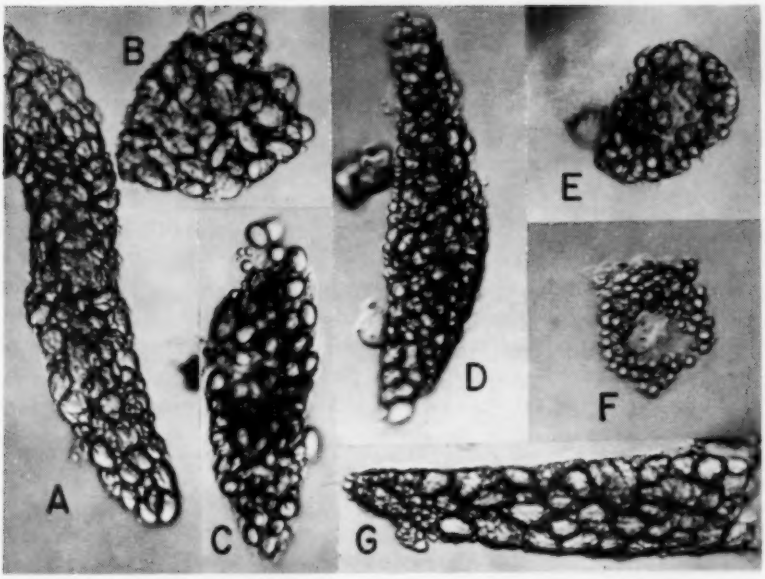

Fig. 2. Endosperm cells from hard wheat flour, showing apparent differences in maturity (× 150).

aleurone of the mature kernel) is the meristematic tissue which produces new endosperm cells during the growth of the kernel (9, 23). In the last portions of the kernel to develop, the cell division of the aleurone continues so late that the growth of these last cells is cut off by the ripening of the kernel—which means that the peripheral cells of the endosperm represent various stages in cell maturity, the particular stage being dependent on the position in the kernel as well as on the field environment during ripening.

Starch Granules. The endosperm cell is packed with starch granules. Cells of the mature type (Fig. 2, A, B, G) contain both large lenticulate granules and small spherical granules. These two types of starch granule, though in the same cell, originated at different periods of cell growth (23); the large granules develop early and are about half grown before the small spherical granules begin to appear. The more immature types of endosperm cells (E, F) contain small to medium-sized lenticular granules, with no small spherical ones. (The space without granules in F is apparently the nucleus.) These small immature lenticular granules may easily be confused with the small (but mature) spherical granules, thus accounting for much of the lack of agreement concerning the properties of the small granules. De-

velopment of cells at C and D was cut off at the stage when granules of the small spherical type were beginning to appear. These wide differences in apparent maturity of endosperm cells and of their constituent starch granules account for many of the variations in characteristics of starch granules.

Protein. The space between starch granules in the endosperm cell is filled with the dried protoplasm of the cell. This dried protoplasm consists largely of protein—the gluten of flour together with soluble constituents (which are removed in washing gluten). The wide variation in characteristics of this protein mixture is responsible for the variation in hardness of the wheat kernel endosperm. In a hard wheat the dried protoplasm is not only hard but, in addition, it forms a strong union with the starch, binding the granules into hard vitreous cells, whereas in the soft wheats it is soft and forms a weak union with the starch.

Cell Walls. The cell walls of the mature endosperm are so weak and friable that they are of little consequence for holding the cell contents together (15, 25). The cell wall is of consequence in milling, however, since it is a weak structure which allows the cells of a hard endosperm to separate from each other without excessive damage to cell contents during milling.

Milling Hard Wheat. Figure 3[4] shows the characteristic break-up of a particle of hard wheat endosperm when subjected to a shearing pressure (25). The elongated endosperm cells of the original particle (Fig. 3, A) tend to separate as individual cells (Fig. 3, B, C, and Fig. 2);

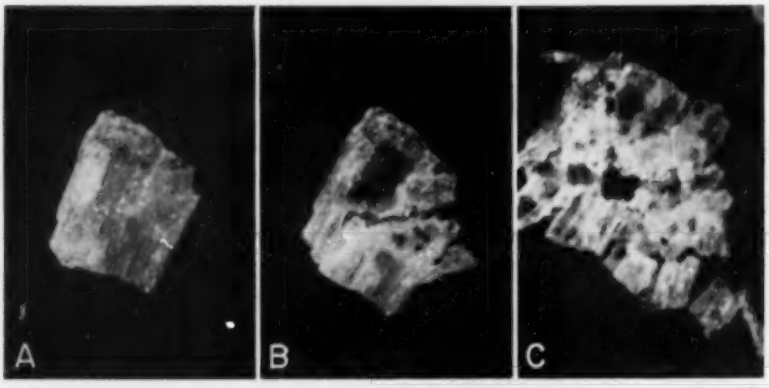

Fig. 3. Breakdown of an endosperm particle from a hard wheat (durum tempered to 15% moisture) under shearing pressure (approx. × 30). (See footnote 4.)

[4] Figures 3 and 4 are enlargements of selected frames from a motion-picture film, showing the effects of endosperm characteristics on milling properties (25).

that is, the breaks tend to follow cell walls. However, in milling, practically all of the cells are damaged to some extent; the starch granules on the cleavage surfaces of the crushed pieces receive severe damage. Also the granules on all surfaces of the cells are given severe abrasive treatment as they go through the rolls and are crushed against other particles. The protein-starch union is so tenacious in this type of endosperm particle that practically no free starch granules are released even when the cell is crushed. Cells and fragments of cells constitute the greater share of flour made from hard wheats (15, 20).

Milling Soft Wheat. In contrast to the hard wheats, in the soft wheats the proteinaceous binding material has little strength and forms a poor union with the starch. Accordingly when a soft wheat endosperm particle is subjected to shearing pressure (Fig. 4) the cells

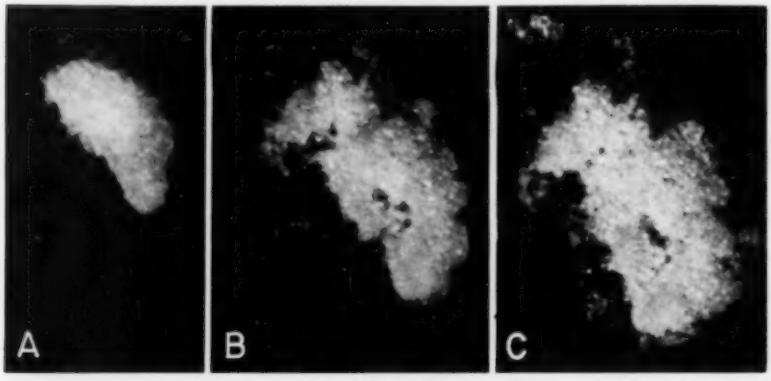

Fig. 4. Breakdown of an endosperm particle from a soft white wheat (Rex, untempered) under shearing pressure (approx. × 30). (See footnote 4.)

readily disintegrate, releasing free but undamaged starch granules (B and C). In this type of endosperm particle, the cell structure is not evident. The resulting flour consists almost entirely of free "protein" particles and free starch granules (25). The starch granules have been subjected to only slight strain and little abrasive action and therefore are not injured.

Characteristics of Damaged Granules

The properties of the damaged starch are of primary importance to the baker; to a large extent the amount of damaged starch determines water absorption, handling properties of doughs, gassing power, and toughness of crumb. Excessive damage may cause poor handling

properties, slackening during fermentation, and poor loaf volume (29)

Water Absorption. The effects of granule damage on starch properties are readily demonstrated by the use of motion-photomicrographic techniques. Since damaged granules swell in cold water and are not likely to regain their original properties or appearance on redrying, the granules to be used for such a study must be prepared from the flour without treatment with water. Those used for this work were prepared by differential settling from petroleum-ether suspensions of a severely damaged commercial flour (a flour that was unsatisfactory because of excessive starch damage).

A drop of a petroleum-ether suspension of the prepared starch is allowed to dry on a microscope slide and a cover glass is added. As observed through the microscope, using transmitted light, the dry starch appears as shown in Fig. 5, A.[5,6] A drop of water is added at

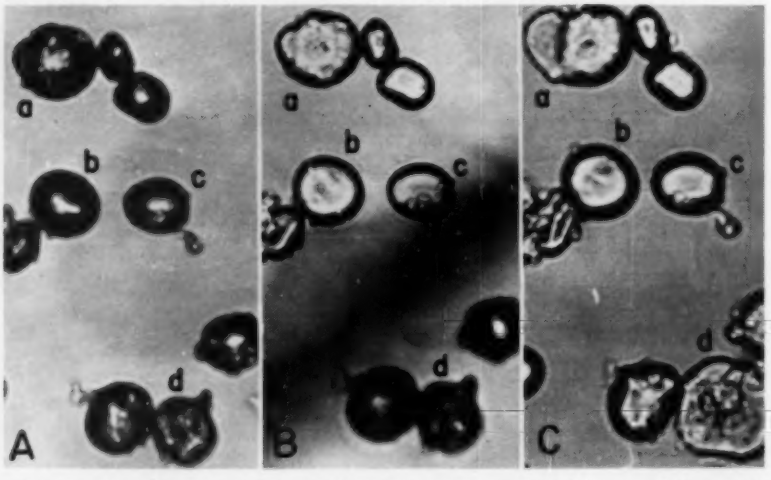

Fig. 5. Absorption of water by mill-damaged starch granules (× 400): A, dry granules. B, field half covered with water; no obvious swelling (water completely crosses the field in about 1/64 second). C, 0.5 second after wetting; swelling apparently complete. (See footnote 6.)

the edge of the cover glass and the camera is started, at 64 frames per second, just before the water flows across the microscopic field of view. Figure 5, B, shows the appearance of the field just as the water has crossed half of the field; the dark area across the field is the advancing

[5] Granules in air appear black. The difference in the index of refraction between starch (1.53) and air (1.0003) allows too little of the light transmitted through the granule to reach the microscope to show more than size and outline.

[6] Figures 5, 6, and 7 are enlargements of selected frames from a motion picture film showing the swelling and digestion of damaged granules (26).

edge of the water. It requires approximately the lapse of time between two frames, 1/64 second, for the water to cross the microscopic field; accordingly, granule *a* had been wet less than 0.016 (1/64) second as shown in Fig. 5, B. A comparison of A and B shows that in this short period there is no evidence of swelling; for comparative purposes, then, B shows the characteristics of the damaged granule before swelling. Figure 5, C, shows the field 30 frames (0.5 second) later. By this time swelling was practically complete.

A normal starch granule when placed in cold water swells to only a limited extent, perhaps 10% in diameter or 30 to 35% in volume (1, 13) (Fig. 5, granules *b* and *c*). On the other hand, in hot water the granule gelatinizes, taking up 10 or more times its weight of water. Supposedly the hydroxyls of a gelatinized starch molecule hold large quantities of water by hydrogen bonding (7). The adsorption of 30 to 35% of water indicates that the raw starch granule is readily permeable to water, yet even after long-continued soaking the swelling does not progress beyond the approximately 35% limit (1). However, if the granule is crushed or otherwise physically injured, it swells in cold water (2) in a manner similar to the swelling of the normal granule in hot water (Fig. 5, granule *d*). This was the enigma of the earlier investigators and it is still a challenging problem.

Damage to the granule does not necessarily extend to all parts (2, 26). It may be a limited local damage, a damage rupturing only a few intermolecular bonds in a small segment (Fig. 5, granule *a*), or it may extend throughout the entire structure (granule *d*). The extensive swelling in water takes place only in the injured portion (Fig. 5, granule *a*). Apparently, however, damage may not only be limited as to area but also as to degree in the area; few or all intermolecular bonds may be ruptured. According to the observable swelling, the damage to granule *a* was not limited to the apparent intensely damaged area to the left but also extends to a lesser degree into the remainder of the granule. The degree of damage may be responsible for wide variations in water absorption in doughs, not only in quantity of water absorbed but also in rate of absorption.

Sectioned Granules. The granules shown in Fig. 6 illustrate another type of damage that occurs quite commonly in the milling of the hardest wheats, such as the durums. As a hard vitreous endosperm cell is broken, granules on the line of cleavage are held so firmly by the protein that they are split in two, a portion of the granule remaining in each piece of the cell (*a* and *b*). The swelling of these two half-granules shows that the internal structure within the pieces may or

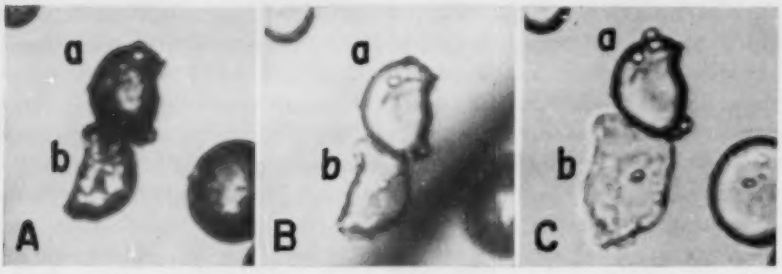

Fig. 6. Absorption of water by granules sectioned by milling (× 400): A, dry granules—sectioned granules a and b; B, sectioned granules wet (probably less than 0.01 second), no swelling; C, one-half second after wetting, swelling complete. (See footnote 6.)

may not be damaged (granule *a* does not swell, whereas *b* swells); the injury to *a* was limited to a very thin layer over the cut surface (2).

Digestion of Damaged Starch. A normal starch granule not only has a limited swelling capacity but is exceedingly resistant to action of digestive enzymes. Gelatinized granules have lost their resistance; as before mentioned, α-amylase action on boiled starch may be from 165 to 7000 times as rapid as on the raw starches. Correspondingly, injured portions of granules have lost their resistance to enzyme action.

By the combination of high-speed and lapsed-time photomicrography, both the swelling and digestion of damaged starches may be shown by flooding the starch with an enzyme solution (26). The swelling is rapid, just as with pure water. The digestion is subsequent to and slower than the swelling; accordingly, after the allowance of 1 second (at 64 frames per second) for completion of swelling, the camera speed is changed to correspond to the slower rate of the subsequent digestion. (This may vary from 8 frames per second to one frame in four or more seconds.)

Figure 7, A, shows a field of mill-damaged granules just after being wetted (before swelling had started) with a 1:5 wheat-malt extract. Swelling was well advanced but incomplete at the end of 0.1 second (B) and apparently complete in 1 second (C) with, as yet, little if any evidence of enzyme action. At the end of 30 minutes (D) the rapid stages of digestion were complete; the almost wholly damaged granule *a* had virtually disappeared, whereas only the locally damaged portions of *b* and *c* had digested. The damaged starch was rapidly digested, whereas the undamaged portions were resistant. The resistance to further digestion of the remaining pieces of partially damaged granules is generally quite variable, some pieces being as resistant as intact uninjured granules. Such evidence indicates that more information con-

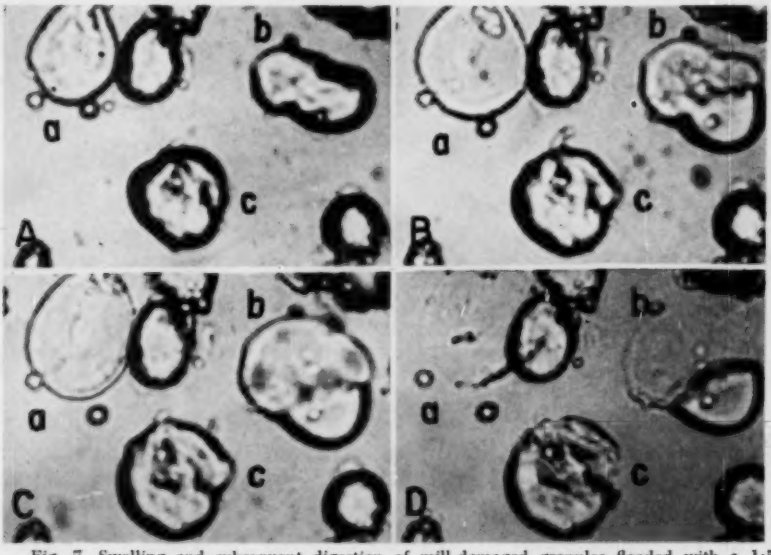

Fig. 7. Swelling and subsequent digestion of mill-damaged granules flooded with a 1:5 malt extract (× 500): A, immediately after wetting (about 1/64 second), before swelling had started; B, 0.1 second after wetting; swelling incomplete; C, 1 second after wetting; swelling complete; D, 30 minutes after wetting; digestion of damaged sections apparently complete. (See footnote 6.)

cerning the internal structure of the granule is necessary before the characteristics of the granule can be satisfactorily explained. The subsequent and much slower attack on the undamaged starch would become evident after longer periods of action.

Beta-Amylase Action on Damaged Starch. Beta-amylase has no action on undamaged starch granules (24, 31); however, it readily digests boiled starch yielding about 60% maltose and residual dextrins. Starch granules that have been severely damaged, e.g., starch that has been subjected to several days of ball-milling, also yields nearly 60% maltose when treated with β-amylase, although the action is very much slower than on boiled starch. Surprisingly, if the action of β-amylase on ball-milled starch is followed by a cursory microscopic examination, little if any change is noticed. However, a comparison of before-and-after photomicrographs shows that though the granules retain their shape and general appearance they become more transparent and considerably smaller.

Figure 8 shows the shrinkage, during the β-amylase action, of granules that had been given a 7-day ball-milling. It should be noted that β-amylase action is not responsible for all of this shrinkage. In the absence of β-amylase a similar shrinkage, although considerably less,

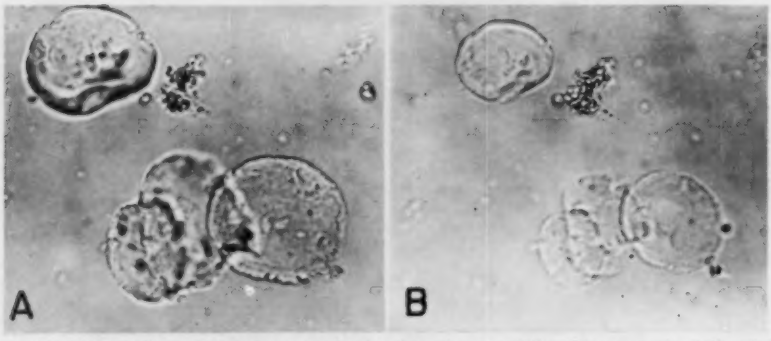

Fig. 8. Digestion of damaged granules by β-amylase (× 400). Wheat starch, ball-milled 7 days, treated with a β-amylase solution: A, about 5 minutes after wetting; B, after 48 hours of digestion.

occurs on mere soaking of such highly damaged granules. This supposedly is due to the solubility of starch molecules and portions of molecules that are freed by the breaking of intermolecular (as well as molecular) bonds (16).

It is noteworthy that wheat starch granules still retain a simulation of granule form after such severe physical punishment. (Under continued grinding the granules eventually are disintegrated.) Even though they have lost all birefringence and as observed in water suspension are highly swollen and transparent, they still retain enough structure to hold together as individual granules; this is similar to the retention of granule individuality in hot-water dispersions.

Grinding of starch is not analogous to grinding other more perfectly crystalline materials. The starch granule is elastic, not rigid. There is accordingly the possibility of rupturing intermolecular (as well as molecular) bonds without complete disintegration of the granule (16). The intermolecular bonds thus freed should be available for hydration (7).

These observations indicate that the degree of injury to the granule structure may vary widely and, correspondingly, granule swelling, solubility, and digestibility (by either α- or β-amylase) also may vary. Since all starches receive a variety of physical treatments during preparation, the many contradictory statements in the literature concerning raw starch properties are understandable.

Enzyme Action on Undamaged Granules

Basic Structure of the Granule. In order to understand the action of the amylases on starch granules it is necessary to have a background knowledge of the basic structure of the granule. Many workers have

attacked the problem of this structure, generally by microscopic observation of structural features as revealed by swelling the granule with chemical agents. Frey-Wyssling (8) and Badenhuizen (3) warn that the features thus shown are artifacts which do not necessarily reflect the original structure, whereas other workers, such as Sponsler (30) and Hansen and Katz (12), believe that, though the structural features were invisible before swelling, the features revealed by the treatment pre-existed in the granule.

The concept of a radially arranged blocklike structure originated from observations by Strasburger in 1882 that radial and tangential cracks formed in granules as they were swollen in potassium hydroxide solutions. If selected observations and portions of the theories of Strasburger, Schimper, Nägeli, A. Meyer, Buscolioni, Bütschli, *et al.* (Reichert's review, 22) were put together we would have essentially the present-day concepts as described by Alsberg (1), Sponsler (30), Hansen and Katz (12), Frey-Wyssling (8), Badenhuizen (3), and Kurt Meyer (19). Though these authors individually do not agree on structural details, their group concept of a granule would seem to be a spherocrystal made up of fiberlike crystals which are oriented both radially and circumferentially (concentrically), the blocklike structure supposedly being due to alternate variation in degree of crystallinity.

As was demonstrated by the action of amylases on injured granules, the structural features of the granule would be expected to determine the rate of amylase action. Accordingly, it should be expected that invisible variations in the structure of the granule may be made visible by the action of the α-amylases. This possibility is indicated in the early literature concerning enzyme action on the starch granule.

Enzyme action on an unaltered wheat or rye starch granule is exceedingly slow and does not take place uniformly throughout the entire granule; accordingly, though such action may show local structural features it does not give a good over-all view of the structure. However, a pretreatment with saturated sodium chloride (or heat, etc.) seemingly permits a rapid and uniform penetration of α-amylase throughout the middle layer of lenticular starch granules, thus allowing a uniform over-all digestion of this layer with the development of a good structural pattern.

Figure 9[7] shows the development of an enzyme action pattern produced by a *Bacillus subtilis* enzyme preparation[8] acting on a rye starch

[7] Figures 9, 13, 14, 15, 17, and 20 are enlargements from a motion picture film showing the structure of the starch granule as revealed by amylase action (27).

[8] Some of the bacterial enzymes, including Bacillus subtilis, malt α-amylase, and the pancreatic and salivary amylases produce similar enzyme action patterns when digesting wheat or rye granules.

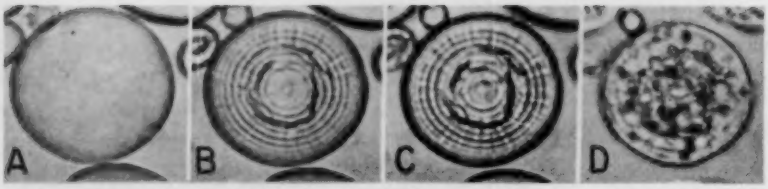

Fig. 9. Over-all structural pattern of a rye starch granule as revealed by Bacillus subtilis amylase action (× 500): A, B, C, progressive stages in the development of the radially and circumferentially oriented structural pattern – the so-called blocklike structure; D, pattern effaced by further digestion. (See footnote 7.)

granule that had had a pretreatment with saturated sodium chloride. Figure 9, A, shows the first faint signs of a pattern. The rings (lamellae) had become clearly visible in B and block structure in C. These patterns disappeared on further digestion (D). Eventually, of course, the granule was completely digested. These patterns indicate that the starch within a granule is not uniform in its susceptibility to enzyme action. Figure 9, C, indicates that the block-structural concept of the starch granule (Hansen and Katz, 12) as a series of concentric rings, each ring made up of "blocks" which are oriented both radially and circumferentially (concentrically), may be correct.

Photomicrographs, being two-dimensional, are a poor medium for conveying a picture of structure and form. However, the third dimension may be introduced if the above granule pattern is visualized as a cross-section of a sphere, each ring of the cross-section representing a sphere. The entire structure, then, would be a series of superimposed concentric spherical layers, each spherical layer consisting of blocks radially oriented with the blocks of the other spheres. Such a spherical granule would be the simplest type which, then, could serve as a model for theorizing on the structure of normal granules of various other shapes. If the model is imagined as being flexible, so as to be capable of being distorted to correspond to the desired shape, the resultant internal structure may be hypothesized.

Shape and Structure of Granules

The granules of wheat, rye, and barley starches are of two distinct types: large lenticular and small spherical (Fig. 2). These granules, though in the same cell, originate at different periods of the cell growth (23). The large granules develop early and attain a diameter of about half that of a mature granule before the small granules begin to appear. The small granules are spherical and range in size from nonobservable to 5μ in diameter; because of Brownian movement granules less than 2μ are not generally observable when suspended

in water. The small granules are of little value for the present study of enzyme action and granule structure, whereas the large granules, because of size and shape, are peculiarly suitable.

The large granules of wheat, rye, and barley are lenticular (shaped like a lentil); round as viewed from above (Fig. 1, *a, c, e*) and oval from the edge with an apparent division between the halves (Fig. 1, *b*). The structure of these granules would correspond to that of the model-granule (Fig. 9) distorted by flattening: the upper and lower sides would be compressed—the rings forced together—whereas the middle layer would be greatly extended, with the rings correspondingly far apart.

A picture of the cross-sectional structure of a wheat starch granule may be obtained by actually sectioning the granules (17) and then following the pattern etched by enzyme action as it develops on the cut surfaces.[9]

Figure 10, A and B, shows the appearance of cross-sectioned granules before enzyme action. The illusory light refraction at the outer rim is disconcertingly realistic in A. Scratches on the cut surface are apparent in B. The extended middle layer of these granules is notable; its prominence could indicate injury during sectioning; on the other hand, it is generally clearly evident in edgewise views of undamaged granules (Fig. 1, *b*).

Two stages in the digestion of cross-sections are shown in Fig. 10, C, D and E, F. Details of the crosswise structure are clearly shown by this digestion; the rings are close together on the short axis, and about twice as far apart and more prominent on the long axis. Each ring is continuous around the granule; that is, it is not interrupted by the extended layer, indicating that the extended layer is a continuation of and may be similar to the rest of the granule except for the greater size of the units. The structural pattern as seen from the flat surface (Fig. 9, C) is the pattern in this middle section of the granule at stages corresponding to C and E.

Figure 10, D and F, shows the granules of C and E after more prolonged digestion. At this stage the structural pattern has been obliterated, corresponding to Fig. 9, D. Apparently the extended middle section is more susceptible to digestion than the more compact layers; supposedly the extended space between the resistant rings in this section contains less resistant starch.

[9] A suspension of granules in gum arabic is sectioned on a freezing microtome. The gum arabic is removed by washing with water by decantation. The starch is dried in a current of air at room temperature. The granules in such a preparation are cut at various angles and may have their internal structure altered. However, good cross sections may be found in the mixture.

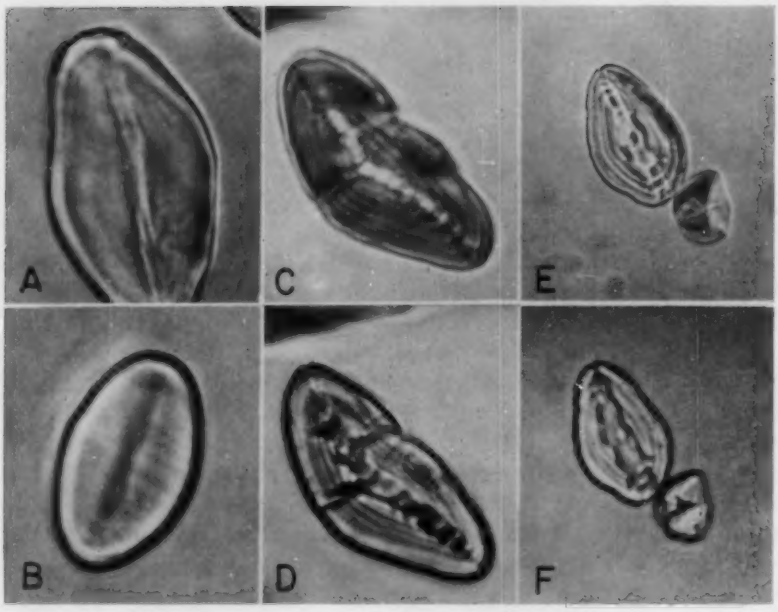

Fig. 10. Amylase action on cross-section granules (× 1800): A, B, cross sectioned granules with no enzyme action, showing the sandwiched middle section; C, D, and E, F, early and late stages in the digestion of the middle section of granules.

When the terms "more resistant" and "less resistant starch" are used, it should not be inferred that these necessarily are different types of starch; theoretically, at least, the differences may be due to differences in orientation of molecules (the degree of crystallinity) and in intermolecular bonding. A number of investigators have suggested that the blocklike structure was a matter of amylose and amylopectin distribution (12). However, the waxy starches (of corn, sorghum, or barley) exhibit a blocklike structure though they contain only amylopectin. (Many waxy granules seemingly contain amylose; however, the above observation applies to apparently pure waxy granules.)

The Digestion of Native (Unaltered) Granules

For the continuous observation of the slow digestion of unaltered granules a small amount of starch (approximately 1 mg.) is placed on a slide with a couple of drops of the enzyme solution.[10] (The enzyme

[10] In general the amylases used for these studies are natural enzyme systems in highly concentrated form; accordingly, we avoid as far as possible the term α-amylase. Purified malt α-amylase gives the pattern of action as shown. However, the other amylases have not been used except as the crude enzyme systems.

solution contains the buffer, 0.2% of calcium chloride, and as a microorganism inhibitor 0.4% of dehydroacetic acid.[11]) A cover glass, with Vaseline-coated edges, is placed over the sample so that an air-tight compartment is formed (18). Digestion is recorded by photomicrographs taken at appropriate intervals or by lapsed-time Cinémicrographs (27). The lapsed-time intervals are adjusted to produce a film that will run about one minute at 24 frames per second, which means that for a 3-day digestion the pictures should be taken at about 3-minute intervals.

Figure 11 shows a series of photomicrographs taken of the action of a 1:5 wheat malt extract on rye starch. Rye starch is used for much of this work because, though the pattern of amylase action on wheat and on rye starch granules is similar, the action is two to three times

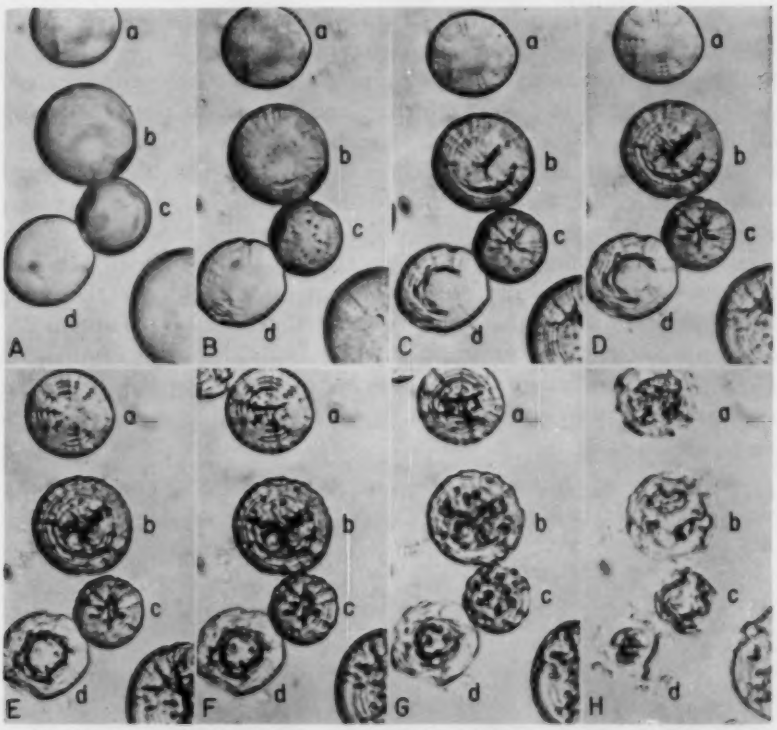

Fig. 11. Malt amylase action on medium-sized rye starch granules (× 350): A-D, progressive stages in the development of the radial pattern, particularly clear in granule a; E, F, further digestion with each digested radial block widening to form the ring-type structure; G, H, further digestion effacing the structural pattern.

[11] Gane's Chemical Works, New York 22, N. Y.

as rapid on rye. Also, rye granules are much larger than those of wheat and accordingly the structural units also are larger and give correspondingly better pictures.

In the amylase action, which seemingly is the more normal, the enzyme enters at the periphery (the narrow edge) and, following the path of least resistance, digests its way into the granule. The first action at the periphery is exceedingly faint, almost invisible (Fig. 11, B, granule *a*). In many cases inability to observe this peripheral entrance of the enzyme has led to misinterpretations of subsequent action (4). The faint lines of action become more pronounced as digestion progresses. As the enzyme follows the path of least resistance, digesting the slightly less resistant starch at a more rapid rate than the more resistant, a temporary pattern is etched from which the structure of the granule may be postulated. The first action of the enzyme on the granules of this particular field (Fig. 11, B and C) was largely radial. This radial pattern of action was particularly clear in granule *a*. As the enzyme crossed each concentric ring (invisible before the action) it had a tendency to follow the ring, and as a result the broadening of the action produced a hole at each ring. The radially oriented series of holes appears to be similar to the "blocks" which are observed when granules are treated with swelling agents. As the subsequent action spreads wider on the rings, the pronounced blocklike appearance becomes definitely ringlike (Fig. 11, E, F, G; granule *a*). The pattern disappears on further action. Subsequent digestion on the outer layers produces a wormeaten appearance (G, H) before complete disappearance of granular substance.

In the field of wheat starch granules shown in Fig. 12, granule *a* shows the radial type of pattern, with each radial line of action ap-

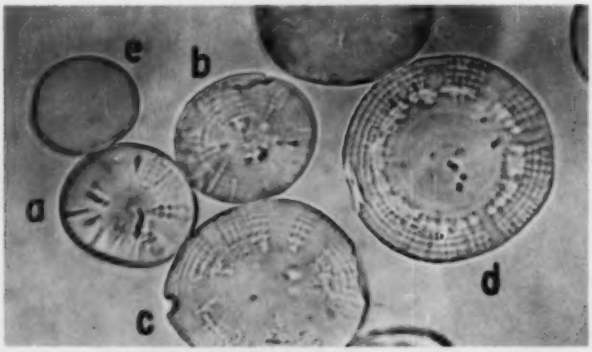

Fig. 12. Structure of the wheat starch granule as revealed by amylase action (× 900).

pearing to be a series of blocks; however, the "blocks" are holes.

Radii converge and meet at the middle of a circle or sphere. Similarly, adjacent radial lines of susceptibility converge and meet, the two lines of action merging into one (as seen in the right-hand side of granule *a*). Since the starch between these converging lines of less resistance must be more resistant, the quantity of resistant starch decreases progressively from the periphery inward until at the last ring before the junction it reaches a minimum. Digestion should be increasingly more rapid as the lines of action converge; the ring containing the least of the resistant starch should be highly susceptible. This type of action is shown in granule *d*. A susceptible ring occurs at that distance in the granule where the adjacent radial lines of susceptibility converge and meet. In the smaller of the large type granule (*a* and *b*) the radial lines extend almost to the middle of the granule, whereas in the larger granules (*c* and *d*) the converging lines of action meet before reaching the middle of the granule, leaving a center island that is more resistant. From the point of view of the development of the granule as contrasted to the view as obtained by destructive digestion, the susceptible ring represents a period in the growth of the granule in which the growth-pattern (and accordingly the crystalline pattern) was changed.

Referring back to Fig. 11, the occurrence of the susceptible ring at the junction of the radial lines of action is illustrated in granules *a*, *b*, and *d*. In granule *c* the radial lines run to the middle.

How does this pattern of enzyme action appear from the edgewise view? Figure 13, A, shows granules with varying stages of digestion as viewed from the flat surfaces. The peculiarly shaped granule *a* has a considerable digested area. Turned on edge (Fig. 13, B, *a*), the action is seen as a hole confined to the extended middle section; the more compact outer layers appear to be intact.

Figure 13, C, shows edgewise views of another group of partially

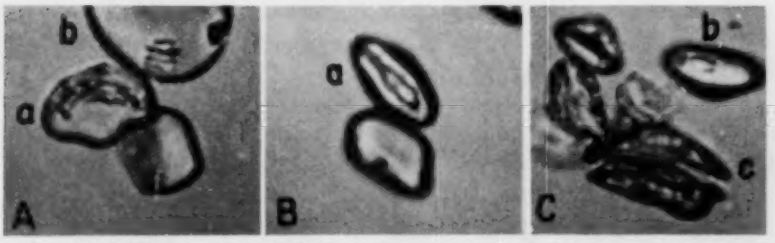

Fig. 13. Edgewise view of amylase action on granules (× 400): A, granules lying flat; B, granule a turned on edge; C, edgewise views of partially digested granules. (See footnote 7.)

digested granules; *b*, with holes along the edge which correspond to early stages of digestion similar to that in granule 13 A, *b*, whereas granule 13 C, *c*, is in an advanced stage of digestion wth the entire middle section gone.

A clearer concept of the over-all action in the interior of the granule may be obtained from the action which takes place on the cut face of a sectioned granule. The half granule shown in Fig. 14 was pro-

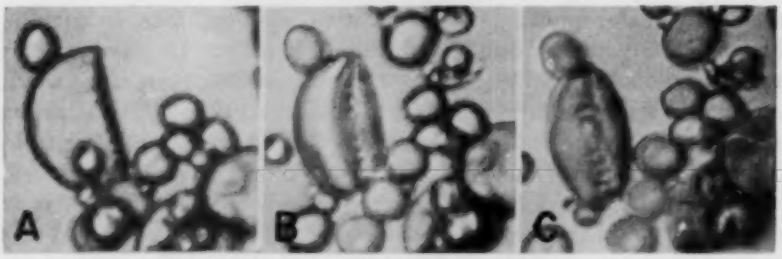

Fig. 14. Amylase action on the cut face of a sectioned granule (× 600): A, partially digested sectioned granule lying on side; B, turned to give a diagonal view of the cut surface; C, turned to give a full view of the pattern of digestion on the cut surface. (See footnote 7.)

duced by damage during milling (compare with Fig. 6). The sawtooth effect of enzyme action on the cut surface (A) indicates digestion of the more susceptible starch from between the resistant rings. As shown in B, this granule has been turned to present a diagonal view which helps to give a three-dimensional concept, and in C, turned further to show the cut face with the indentations digested along the extended axis. All evidence of action at this stage is confined to this susceptible layer. Further evidence of the structural features of the interior of the granule may be obtained by referring back to Fig. 10 which shows cross-sectioned granules after enzyme action. This is the type of cross-sectional structure that would be expected; the resistant rings of necessity are far apart on the long axis. On the other hand, the outer layers are compact. These compact outer layers apparently contain a minimum of the more susceptible starch and are correspondingly resistant to enzyme action, whereas the middle layer, where the resistant layers are far apart, is correspondingly susceptible.

It is apparent that the structural features that have been shown are the features of the more susceptible middle layer of the granule. This is the section where the structural features are naturally magnified and clearly defined. With more rapid digestion, the pattern of digestion develops early, offering a structural picture uncontaminated by patterns in the layers above or below. Projecting the structure of

this layer into the more resistant outer layers involves the assumption that these are similar except for the size of the units.

The course of digestion of the larger wheat and rye starch granules is further illustrated in Fig. 15. As shown in A, digestion had started

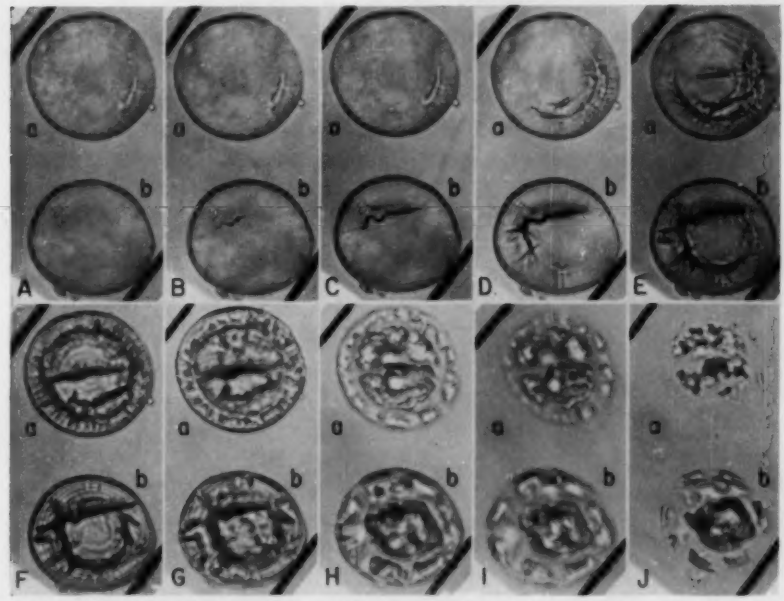

Fig. 15. Action of malt amylases on large rye starch granules (× 350): A, radial action from the periphery; B-E, progressive digestion of the susceptible ring and cracks in the granule; F-J, further digestion showing residual structures (the more resistant portions of the granule) as found in the late stages of amylase action. (See footnote 7.)

at the periphery and, following a narrow radial path in granule *a* and a wider path in *b*, had reached the susceptible ring. The path of action at the periphery is almost invisible, the digested pattern becoming more distinct wth progress inward. At the susceptible ring the action in granule *a* follows the ring from which it spreads both inward and outward (D and E), reinforced by other action from the periphery.

If the granules at the stage shown in G were turned to give an edgewise view, they would be similar to the granule shown in Fig. 16. The extended middle section appears to have been completely digested. It is possible that the lines of action (*a* and *b*) perpendicular to the middle section are due to the development of cracks as shown in Fig. 15, granule *b*. It is evident that the two halves of such a digested granule (Fig. 16) would readily separate if the digest were stirred and could be confusing if found in a catch sample.

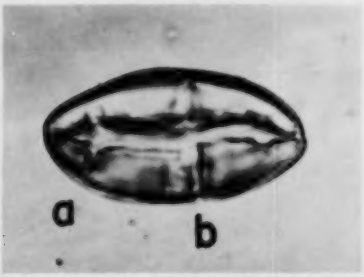

Fig. 16. Edgewise view of a partially digested granule (× 600).

The pattern of action on the more resistant middle piece that remains after complete digestion of the susceptible ring is not clear. In most cases digestion causes the middle piece to split (granule *a* of Fig. 15, E and F) as described in the next section. It is assumed that the structure of this middle piece of the large granule is similar to the structure of smaller granules (Fig. 12, granules *a*, *b*). Its resistance to amylase action is to be expected since the structure of its outer rings should be similar to that in the outer rings of the smaller granules.

The greater resistance of the peripheral rings is evident (Fig. 15, H, I, J). The remnants of these outer layers could easily be misinterpreted as evidence of a granule membrane if their formation during digestion had not been followed by continuous observation.

Variations in Pattern of Digestion. Although the pattern of amylase action as shown above is designated as the more "normal" action, other patterns commonly occur and since these are of frequent occurrence they indicate normal variations in the structure of the granule. Usually these variations are superimposed on the more normal pattern, being additional variants.

The digestion of granule *b* of Fig. 15 shows one of these commonly occurring variations; the enzyme enters at the periphery in a normal manner; however, at about the location of the usual susceptible ring, the enzyme action causes a split (or crack) to develop across the granule. Supposedly such splitting action may indicate a flaw in the granule structure which would permit a small local swelling, the consequent pressure causing the beginning rupture, the rupture allowing further swelling and further rupture, etc., across the granule. The rupture generally is not instantaneous but usually requires a few seconds to possibly hours for completion. The subsequent enzyme action may spread from the resulting fissure; in some cases, however, the newly exposed surfaces are little, if any, more susceptible than un-

injured starch in a similar position in the granule. The beginning of a fissure and its development is shown in Fig. 15, B, C, D, E, F.

Another frequently observed pattern of action was shown in granule *a* of Fig. 1. This granule shows not only the "normal" action developing from the periphery but a pitting action on the ordinarily resistant upper and lower surfaces. Apparently in some granules these surfaces are no more resistant than the periphery of the middle layer. Each hole (or dot) as shown in A and C represents the end view of a radial line of action. Other examples of this pitting type of action are shown in granules *b* and *c* of Fig. 11, B, and in granule *d* of Fig. 12.

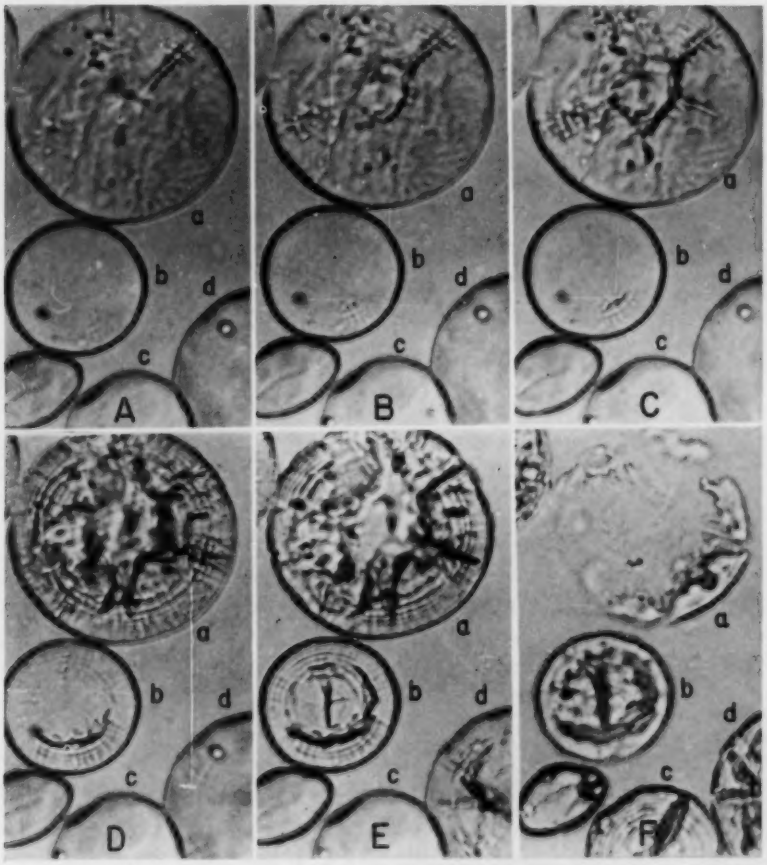

Fig. 17. Susceptible middle section in a rye starch granule: A, digestion begins with radial action from the periphery; B-F, progressive stages in the digestion. (See footnote 7.)

Granule *a* of Fig. 17 shows still another type of digestion. The enzyme enters at the periphery but the entire middle section is susceptible and is rapidly digested. Granules injured by pressure on the cover glass frequently digest in this way. Accordingly this could be an injured granule. However, evidence that this may be an uninjured granule is shown by the occurrence of this type of granule in isolated endosperm cells (Fig. 19, granule cluster *G*).

The granules of Fig. 17 also illustrate other types of action: granule *b* shows typical action, the radially oriented holes giving a good blocklike structural picture in the outer rings with a splitting middle piece. Granules *c* and *d* show the splitting pattern of action.

Variations in Susceptibility. The rate of digestion varies widely from granule to granule. The granules of Fig. 17 may be cited as examples: Compare the action on granules *a* and *b* with the late initiation of action on granule *c* and the still slower action on granule *d*.

Digestion of Immature Granules. Various authors have suggested that the rings and blocklike structures observed in granules may be the result of stresses set up in the granules during drying. However, these structural features are evident in immature wheat starch granules—granules washed from wheat at the soft-dough stage and subjected to malt enzyme action without having been subjected to any drying procedure (Fig. 18).

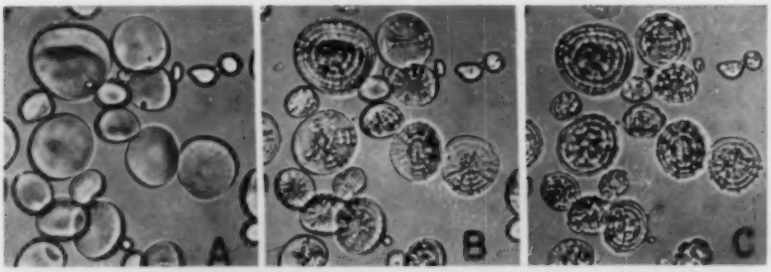

Fig. 18. Pattern of action of malt amylases on immature wheat starch which had not been dried (× 500): A, faint pattern of early action; B, C, further stages of action.

Immature granules are more susceptible to enzyme action than mature ones. However, the patterns of action are quite similar and indicate similar structural features. As a general rule in such small immature granules the converging radial lines of amylase action meet at the middle of the granule. Since both the immature granules and the relatively small mature granules (Fig. 12, *a* and *b*) have this structure extending to the middle of the granule, as before stated, it is

assumed that the middle pieces of the large mature granules (Fig. 15, G) also would have the same structure.

Origin of Structural Differences. Since it seems that the differences in the pattern of action of malt enzymes on starch granules indicate differences in granule structure, it is of interest to know the origin of the differences. Though there are variations in predominance of types of granules from one kernel of wheat to another, the starch from a single kernel will generally show all of the various types of amylase action patterns. However, the action on all of the granules from an individual endosperm cell seems to be of a single pattern. For illustration, individual cells were separated from a hard wheat flour (as shown in Fig. 2) and sealed under cover glasses on slides with a 1:10 malt extract. A preliminary soaking period of about 15 minutes was allowed for softening the gluten so as to permit easy disintegration of the cells by gentle manipulation of the cover glass. Some cross contamination from one cell to another is probable and accordingly an occasional out-of-place granule is to be expected.

Photomicrographs were taken of selected fields—fields on the slide that showed neighboring cells with widely varying characteristics. Figure 19 shows selected fields from three slides, all prepared from the same enzyme solution at approximately the same time; each segregated cluster of granules represents an individual cell. All of the granules from any particular cell appear to be similar in respect to the pattern

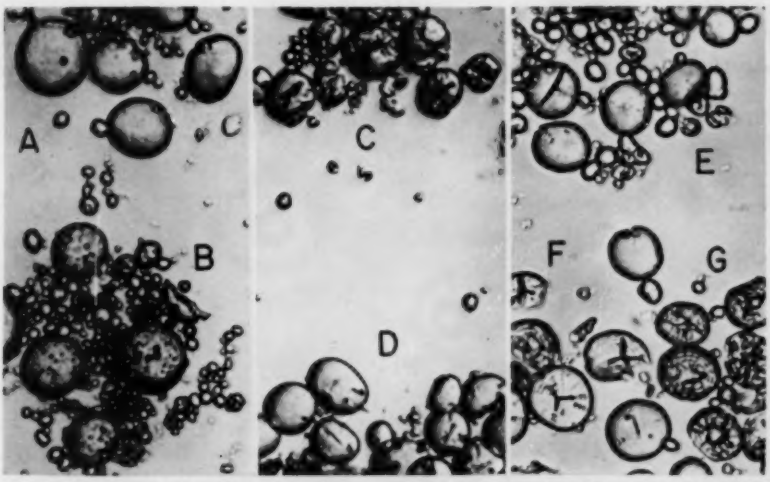

Fig. 19. Comparison of enzyme action on granules from isolated endosperm cells (× 250). Each labeled cluster of granules represents an individual endosperm cell. Note the similarity of granules from an individual cell.

of action of malt α-amylase but may differ widely from those of other cells. Granules from cell *A* were resistant to amylase action; those from *B* were susceptible to the pitting type of action; those from *C* also were susceptible but to a splitting action; the granules from *D* were relatively resistant, seemingly with a potential splitting type of action; those from *E* were resistant with the predominant pattern still questionable; *F* showed the more commonly observed, well-defined action from the periphery with a tendency for the middle piece to split (some of these granules appear to have been damaged); whereas, the granules from *G* all had susceptible middle sections (similar to that of granule *a* in Fig 17).

This series of disintegrated endosperm cells, each with granules showing one of the characteristic variations in the pattern of amylase action, indicates that generally these variations in pattern are not due to mechanical injury to the granules but to structural variations produced by environmental conditions in the growing cell.

Differences between Enzymes from Different Sources

It is well known that the α-amylases from such varied sources as cereals, fungi, bacteria, and the digestive juices of animals differ in many respects: optimum pH for activity, stability to heat, etc. It has recently been shown that they differ greatly in the rate at which they attack the raw starch granule (28); accordingly it is not surprising that they also differ in the manner of their attack on raw starch.[12] Pancreatic, salivary, and some of the bacterial amylases follow the same pattern as shown for malt α-amylase. However, other fungals and bacterials have distinct patterns of the pitting type.

Figure 20 shows the pattern of action of the enzyme system of *Aspergillus oryzae* on rye starch. This enzyme system does not differ-

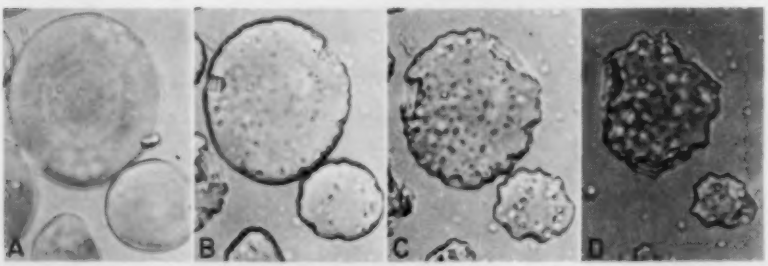

Fig. 20. Progressive stages in the pattern of action of an Aspergillus oryzae enzyme system on rye starch (× 500). (See footnote 7.)

[12] See footnote 10, page 32.

entiate as clearly as malt between the resistant and less resistant blocks or rings of the granule. Digestion is somewhat faster at the periphery than from the other surfaces, but it does not follow either the radial or ring type of pattern. Insofar as this enzyme is concerned, there is no susceptible ring. However, the pitted surface indicates differences in susceptibility. The enzyme apparently does not penetrate the granule deeply but digests the starch by widening the shallow pits. No cracks occur in the granule during this action.

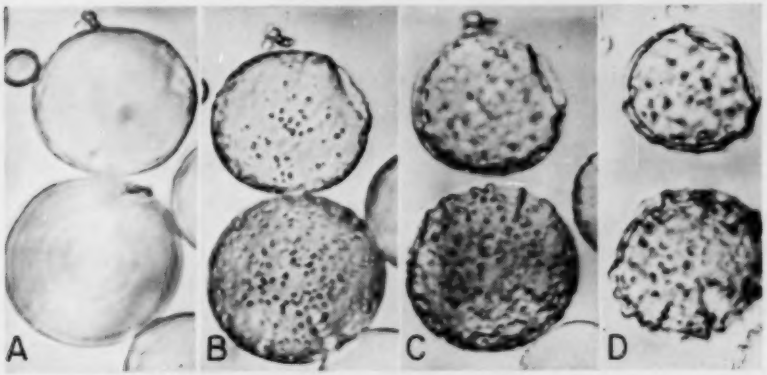

Fig. 21. Progressive stages in the pitting pattern of action of another preparation of Aspergillus oryzae on rye granules (× 600).

Figure 21 shows digestion by another preparation of *Aspergillus oryzae* which has a more clear-cut pitting type of action with little or no preference for the extended middle layer of the granule or for the rings which were so susceptible to the malt amylases.

These differences in action indicate that the weak points in the starch granules, insofar as one enzyme is concerned, are not necessarily the weak points for other enzymes. Whether these indicate differences in the attack on the starch molecule or merely in the ability of the enzyme to penetrate areas of a particular structure remains to be seen.

Summary

Normally, wheat starch granules are subjected to various types of damage during milling: crushing, tearing, and abrasion. These lead to many types of injury, some of which were shown in Figs. 5, 6, and 7. Figure 22, malt amylase action on a wheat starch that had been ball-milled for one hour, is presented as a summary and as a means of bringing together into one picture the rapid swelling and digestion

of damaged starch in comparison to the very slow subsequent digestion of undamaged portions of the granule.

Figure 22, A, illustrates the distinctly local character of the water

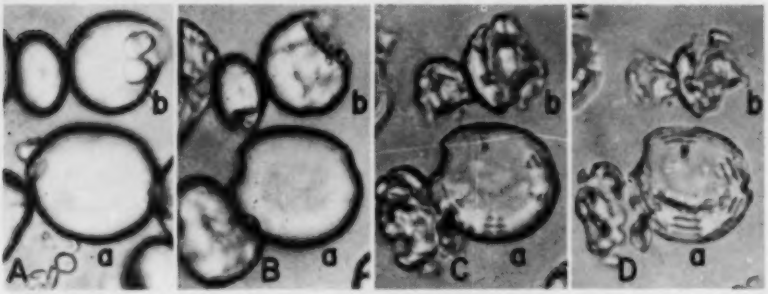

Fig. 22. Amylase action on injured starch granules (× 600): A, suspended in 1:20 malt extract, about 5 minutes after wetting; B, after one hour; C, after 24 hours; D, after 48 hours.

absorption that takes place in injured portions of granules. Digestion of this highly swollen damaged starch was apparently complete before the end of the first hour (B). As with the swelling, the rapid digestion was limited to the areas of damage. In the case of a small local injury, such as that on granule *a*, digestion was confined to the local point of injury and did not extend farther inward. On the other hand, an injury may involve much or all of the granule (Figs. 5, 6, 7). In granule *b* the injury was somewhat ramified throughout the granule.

The resistance to further digestion of the irregularly shaped pieces of granules remaining after digestion of the damaged starch is similar to the resistance of normal undamaged granules. The local damaged area of granule *a* was removed during the first hour's digestion; the surface thus exposed was similar to undamaged portions of the rest of the periphery in its resistance to enzyme action—there was no digestion from this surface during the next 23 to 48 hours, whereas radial action followed by widespread tangential digestion was notable from other areas (C, D).

Since the microscopic pattern etched by enzyme action during digestion of the granule is due to slight differences in resistance to enzyme action, this offers a tool for bringing to view many otherwise invisible structural features, especially if the entire course of digestion is studied from various angles.

Figure 23 is shown as a brief summary of structural features as revealed by amylase action. Granule *a* shows an area in which the blocklike oriented structure is apparent; *b* indicates how this area of

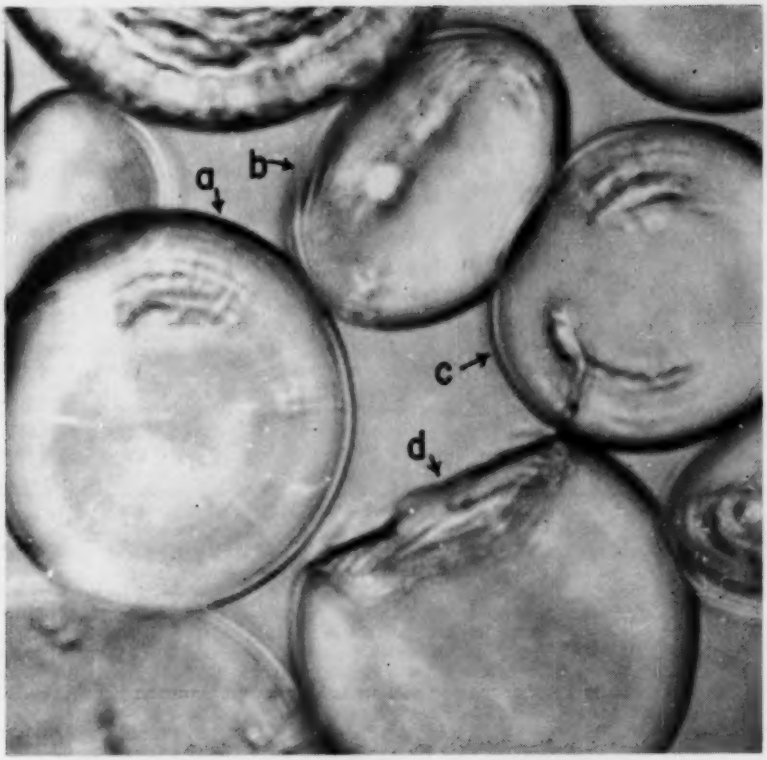

Fig. 23. Structure as revealed by a field of digesting granules (× 1500): a, Area of block-like structure; b, edgewise view of the action; c, variation of susceptibility in the granule, the susceptible ring; d, the layered structure as shown by enzyme action on a sectioned granule.

digestion would appear if *a* were turned on edge; *c* shows the variation in susceptibility within the granule (the susceptible ring); and *d* shows the onionlike layering as revealed by amylase action on a cross-sectioned face of a granule. If these views could be combined the resulting composite picture would represent our present microscopic concept of the wheat and rye starch granule.

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